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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : **C12N 15/12, C07K 14/51, A61K 38/18**	**A1**	(11) International Publication Number: **WO 95/33830** (43) International Publication Date: 14 December 1995 (14.12.95)

(21) International Application Number: PCT/US95/07084

(22) International Filing Date: 5 June 1995 (05.06.95)

(30) Priority Data:
08/254,353   6 June 1994 (06.06.94)   US

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(81) Designated States: AU, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BMP-9 COMPOSITIONS

(57) Abstract

    Purified BMP-9 proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and cartilage defects and in wound healing and related tissue repair, and in hepatic growth and function.

BMP-9 COMPOSITIONS

The present invention relates to a novel family of purified proteins designated BMP-9 proteins and processes for obtaining them. These proteins may be used to induce bone and/or cartilage

5   formation, in wound healing and tissue repair, and in hepatic growth and function.

The murine BMP-9 DNA sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) are set forth in Figure 1. Human BMP-9 sequence is set forth in Figure 3 (SEQ ID NO:8 and SEQ ID NO:9).

10   It is contemplated that BMP-9 proteins are capable of inducing the formation of cartilage and/or bone. BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

15   Murine BMP-9 is characterized by comprising amino acid #319 to #428 of Figure 1 (SEQ ID NO:2 amino acids #1-110). Murine BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #610 to nucleotide #1893 as shown in Figure 1 (SEQ ID NO:1) and recovering and purifying from the

20   culture medium a protein characterized by the amino acid sequence comprising amino acid #319 to #428 as shown in Figure 1 (SEQ ID NO:2) substantially free from other proteinaceous materials with which it is co-produced.

Human BMP-9 is expected to be homologous to murine BMP-9 and

25   is characterized by comprising amino acid #1 (Ser, Ala, Gly) to #110 of Figure 3 (SEQ ID NO:9) (Arg). The invention includes methods for obtaining the DNA sequences encoding human BMP-9. This method entails utilizing the murine BMP-9 nucleotide sequence or portions thereof to design probes to screen libraries

30   for the human gene or fragments thereof using standard techniques. Human BMP-9 may be produced by culturing a cell transformed with the BMP-9 DNA sequence and recovering and purifying BMP-9 from the culture medium. The expressed protein is isolated, recovered, and purified from the culture medium.

The purified expressed protein is substantially free from other proteinaceous materials with which it is co-produced, as well as from other contaminants. The recovered purified protein is contemplated to exhibit cartilage and/or bone formation activity. The proteins of the invention may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO:8 and recovering and purifying from the culture medium a protein characterized by the amino acid sequence of SEQ ID NO:9 from amino acid #1 to amino acid #110 substantially free from other proteinaceous materials with which it is co-produced.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle or carrier. BMP-9 compositions of the invention may be used in the formation of cartilage. These compositions may further be utilized for the formation of bone. BMP-9 compositions may also be used for wound healing and tissue repair. Compositions of the invention may further include at least one other therapeutically useful agent such as the BMP proteins BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, and BMP-7 disclosed for instance in PCT Publication Nos. WO88/00205, WO89/10409, and WO90/11366, and BMP-8, disclosed in U.S. Application Serial No. 07/641,204 filed January 15, 1991, Serial No. 07/525,357 filed May 16, 1990, and Serial No. 07/800,364 filed November 20, 1991.

The compositions of the invention may comprise, in addition to a BMP-9 protein, other therapeutically useful agents including growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF-α and TGF-β), and insulin-like growth factor (IGF). The compositions may also include an appropriate matrix for instance, for supporting the composition and providing a surface for bone and/or cartilage growth. The matrix may provide slow release of the osteoinductive protein and/or the appropriate environment for presentation thereof.

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The BMP-9 compositions may be employed in methods for treating a number of bone and/or cartilage defects, periodontal disease and various types of wounds. These methods, according to the invention, entail administering to a patient needing such bone and/or cartilage formation wound healing or tissue repair, an effective amount of a BMP-9 protein. These methods may also entail the administration of a protein of .the invention in conjunction with at least one of the novel BMP proteins disclosed in the co-owned applications described above. In addition, these methods may also include the administration of a BMP-9 protein with other growth factors including EGF, FGF, TGF-α, TGF-β, and IGF.

Still a further aspect of the invention are DNA sequences coding for expression of a BMP-9 protein. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Figure 1 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:8) or DNA sequences which hybridize under stringent conditions with the DNA sequences of Figure 1 or 3 and encode a protein having the ability to induce the formation of cartilage and/or bone. Finally, allelic or other variations of the sequences of Figure 1 or 3, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present invention.

A further aspect of the invention includes vectors comprising a DNA sequence as described above in operative association with an expression control sequence therefor. These vectors may be employed in a novel process for producing a BMP-9 protein of the invention in which a cell line transformed with a DNA sequence encoding a BMP-9 protein in operative association with an expression control sequence therefor, is cultured in a suitable culture medium and a BMP-9 protein is recovered and purified therefrom. This process may employ a number of known cells both prokaryotic and eukaryotic as host cells for expression of the polypeptide.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 comprises DNA sequence and derived amino acid sequence of murine BMP-9 from clone ML14a further described below.

FIG. 2 comprises DNA sequence and derived amino acid sequence of
5   human BMP-4 from lambda U2OS-3 ATCC #40342.

FIG. 3 comprises DNA sequence and derived amino acid sequence of human BMP-9 from λ FIX/H6111 ATCC #75252.

FIG. 4 sets forth articular cartilage assay sulfate incorporation results.

10   FIG. 5 sets forth results of specific BMP-9 binding to HepG2 cells.

FIG. 6 sets forth results of stimulation of HepG2 cell proliferation by BMP-9.

FIG. 7 sets forth the results of stimulation of primary rat
15   hepatocytes by BMP-9.

DETAILED DESCRIPTION OF THE INVENTION

The murine BMP-9 nucleotide sequence (SEQ ID NO:1) and encoded amino acid sequence (SEQ ID NO:2) are depicted in Figure 1. Purified murine BMP-9 proteins of the present invention are
20   produced by culturing a host cell transformed with a DNA sequence comprising the DNA coding sequence of Figure 1 (SEQ ID NO:1) from nucleotide #610 to nucleotide #1893 and recovering and purifying from the culture medium a protein which contains the amino acid sequence or a substantially homologous sequence as represented
25   by amino acid #319 to #428 of Figure 1 (SEQ ID NO:2). The BMP-9 proteins recovered from the culture medium are purified by isolating them from other proteinaceous materials from which they are co-produced and from other contaminants present.

Human BMP-9 nucleotide and amino acid sequence is depicted
30   in SEQ ID No: 8 and 9.   Mature human BMP-9 is expected to comprise amino acid #1 (Ser, Ala, Gly) to #110 (Arg).

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO:8 and recovering and purifying from the culture
35   medium a protein characterized by the amino acid sequence of SEQ ID NO:9 from amino acid #1 to amino acid #110 substantially free

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from other proteinaceous materials with which it is co-produced.

BMP-9 proteins may be characterized by the ability to induce the formation of cartilage. BMP-9 proteins may be further characterized by the ability to induce the formation of bone.

5   BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

The BMP-9 proteins provided herein also include factors encoded by the sequences similar to those of Figure 1 and 3 (SEQ

10   ID NOS:1 and 8), but into which modifications are naturally provided (e.g., allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino

15   acid residues of Figure 1 of Figure 3 (SEQ ID NOS:2 and 9). These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Figure 1 and Figure 3 may possess bone growth factor biological properties in common therewith.

20   Thus, they may be employed as biologically active substitutes for naturally-occurring BMP-9 and other BMP-9 polypeptides in therapeutic processes.

Other specific mutations of the sequences of BMP-9 proteins described herein involve modifications of glycosylation sites.

25   These modifications may involve O-linked or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at asparagine-linked glycosylation recognition sites. The asparagine-linked glycosylation

30   recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one

35   or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified

tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for BMP-9 proteins. These DNA sequences include those depicted in Figure 1 or Figure 3 (SEQ ID NOS:1 and 8) in a 5' to 3' direction and those sequences which hybridize thereto under stringent hybridization conditions [see, T. Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] and encode a protein having cartilage and/or bone inducing activity.

Similarly, DNA sequences which code for BMP-9 proteins coded for by the sequences of Figure 1 or Figure 3, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel factors described herein. Variations in the DNA sequences of Figure 1 or Figure 3 (SEQ ID NOS:1 and 8) which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing BMP-9 proteins. The method of the present invention involves culturing a suitable cell line, which has been transformed with a DNA sequence encoding a BMP-9 protein of the invention, under the control of known regulatory sequences. The transformed host cells are cultured and the BMP-9 proteins recovered and purified from the culture medium. The purified proteins are substantially free from other proteins with which they are co-produced as well as from other contaminants.

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening, product production and purification are known in the art. See e.g., Gething and Sambrook, Nature 293:620-625 (1981), or alternatively, Kaufman et al., Mol. Cell.

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Biol. 5(7):1750-1759 (1985) or Howley et al., U.S. Patent No. 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable.

5   Bacterial cells may also be suitable hosts. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

10   Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See e.g., Miller et al., Genetic

15   Engineering 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel BMP-9 polypeptides. Preferably the vectors contain the full novel DNA

20   sequences described above which encode the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the BMP-9 protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present

25   invention. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Regulatory sequences

30   for such vectors are known to those skilled in the art and may be selected depending upon the host cells. Such selection is routine and does not form part of the present invention.

A protein of the present invention, which induces cartilage and/or bone formation in circumstances where bone is not normally

35   formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a BMP-9 protein may have prophylactic use

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in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced

5   craniofacial defects, and also is useful in cosmetic plastic surgery. A BMP-9 protein may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation

10   of progenitors of bone-forming cells. BMP-9 polypeptides of the invention may also be useful in the treatment of osteoporosis. BMP-9 may be used in cartilage defect repair and prevention/ reversal of osteoarthritis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been

.15 described. See e.g., European Patent Application Nos. 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See e.g.,

20   PCT Publication No. WO84/01106 for discussion of wound healing and related tissue repair).

It is further contemplated that proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease

25   in neuronal survival.

BMP-9 proteins of the invention may also be useful in hepatic growth and function including repair and regeneration of liver cells. BMP-9 may therefore be used for instance in treatment of conditions exhibiting degeneration of the liver.

30   A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal dis-eases. The invention further comprises therapeutic methods and compositions for wound healing and tissue repair. Such

35   compositions comprise a therapeutically effective amount of at least one of the BMP-9 proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix.

It is expected that the proteins of the invention may act in concert with or perhaps synergistically with other related proteins and growth factors. Further therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of at least one BMP-9 protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned applications described above. Such combinations may comprise separate molecules of the BMP proteins or heteromolecules comprised of different BMP moieties. For example, a method and composition of the invention may comprise a disulfide linked dimer comprising a BMP-9 protein subunit and a subunit from one of the "BMP" proteins described above. A further embodiment may comprise a heterodimer of BMP-9 moieties. Further, BMP-9 proteins may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with BMP-9 of the present invention.

The therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the BMP-9 proteins which may also optionally be included in the

composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the BMP composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would

5   include a matrix capable of delivering BMP-9 or other BMP proteins to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. The matrix may provide slow release of BMP-9 and/or the appropriate environment for

10   presentation thereof. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the BMP-9

15   compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or

20   dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above

25   mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

30   The dosage regimen will be determined by the attending physician considering various factors which modify the action of the BMP-9 protein, e.g., amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's

35   age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the types

of BMP proteins in the composition. The addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair,
5   for example, x-rays, histomorphometric determinations and tetracycline labeling.

The following examples illustrate practice of the present invention in recovering and characterizing murine BMP-9 protein and employing it to recover the human and other BMP-9 proteins,
10   obtaining the human proteins and expressing the proteins via recombinant techniques.

EXAMPLE I

MURINE BMP-9

750,000 recombinants of a mouse liver cDNA library made in
15   the vector lambdaZAP (Stratagene/Catalog #935302) are plated and duplicate nitrocellulose replicas made. A fragment of human BMP-4 DNA corresponding to nucleotides 1330-1627 of Figure 2 (SEQ ID NO:3) (the human BMP-4 sequence) is ^{32}P-labeled by the random priming procedure of Feinberg et al., Anal. Biochem. 132:6-13
20   (1983) and hybridized to both sets of filters in SHB at 60°C for 2 to 3 days. Both sets of filters are washed under reduced stringency conditions (4X SSC, 0.1% SDS at 60°C). Many duplicate hybridizing recombinants of various intensities (approximately 92) are noted. 50 of the strongest hybridizing recombinant
25   bacteriophage are plaque purified and their inserts are transferred to the plasmid Bluescript SK (+/-) according to the in vivo excision protocol described by the manufacturer (Stratagene). DNA sequence analysis of several recombinants indicate that they encode a protein homologous to other BMP
30   proteins and other proteins in the TGF-β family. The DNA sequence and derived amino acid sequence of one recombinant, designated ML14a, is set forth in Figure 1. (SEQ ID NO:1)

The nucleotide sequence of clone ML14a contains an open reading frame of 1284 bp, encoding a BMP-9 protein of 428 amino
35   acids. The encoded 428 amino acid BMP-9 protein is contemplated to be the primary translation product as the coding sequence is

preceded by 609 bp of 5' untranslated sequence with stop codons
in all three reading frames. The 428 amino acid sequence
predicts a BMP-9 protein with a molecular weight of 48,000
daltons.

5   Based on knowledge of other BMP proteins and other proteins
within the TGF-β family, it is predicted that the precursor
polypeptide would be cleaved at the multibasic sequence ARG-ARG-
LYS-ARG in agreement with a proposed consensus proteolytic
processing sequence of ARG-X-X-ARG. Cleavage of the BMP-9
10   precursor polypeptide at this location would generate a 110 amino
acid mature peptide beginning with the amino acid SER at position
#319. The processing of BMP-9 into the mature form is expected
to involve dimerization and removal of the N-terminal region in
a manner analogous to the processing of the related protein TGF-β
15   [L. E. Gentry et al., Mol. & Cell. Biol. 8:4162 (1988); R.
Derynck et al., Nature 316:701 (1985)].

         It is contemplated therefore that the mature active species
of murine BMP-9 comprises a homodimer of 2 polypeptide subunits,
each subunit comprising amino acids #319-#428 with a predicted
20   molecular weight of approximately 12,000 daltons. Further active
species are contemplated comprising amino acids #326 - #428
thereby including the first conserved cysteine residue. As with
other members of the BMP and TGF-β family of proteins, the
carboxy-terminal region of the BMP-9 protein exhibits greater
25   sequence conservation than the more amino-terminal portion. The
percent amino acid identity of the murine BMP-9 protein in the
cysteine-rich C-terminal domain (amino acids #326 - #428) to the
corresponding region of other human BMP proteins and other
proteins within the TGF-β family is as follows: BMP-2, 53%; BMP-
30   3, 43%; BMP-4, 53%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; Vg1, 50%;
GDF-1, 43%; TGF-β1, 32%; TGF-β2, 34%; TGF-β3, 34%; inhibin β(B),
34%; and inhibin β(A), 42%.

                                EXAMPLE II

HUMAN BMP-9
35   Murine and human osteoinductive factor genes are presumed
to be significantly homologous, therefore the murine coding

sequence or a portion thereof is used as a probe to screen a human genomic library or as a probe to identify a human cell line or tissue which synthesizes the analogous human cartilage and/or bone protein. A human genomic library (Toole et al., supra) may

5   be screened with such a probe, and presumptive positives isolated and DNA sequence obtained. Evidence that this recombinant encodes a portion of the human BMP-9 relies of the murine/human protein and gene structure homologies.

Once a recombinant bacteriophage containing DNA encoding

10   portion of the human cartilage and/or bone inductive factor molecule is obtained, the human coding sequence can be used as a probe to identify a human cell line or tissue which synthesizes BMP-9. Alternatively, the murine coding sequence can be used as a probe to identify such human cell line or tissue. Briefly

15   described, RNA is extracted from a selected cell or tissue source and either electrophoresed on a formaldehyde agarose gel and transferred to nitrocellulose, or reacted with formaldehyde and spotted on nitrocellulose directly. The nitrocellulose is then hybridized to a probe derived from a coding sequence of the

20   murine or human BMP-9. mRNA is selected by oligo (dT) cellulose chromatography and cDNA is synthesized and cloned in lambda gt10 or lambda ZAP by established techniques (Toole et al., supra).

Additional methods known to those skilled in the art may be used to isolate the human and other species' BMP-9 proteins of

25   the invention.

A.   ISOLATION OF HUMAN BMP-9 DNA

One million recombinants of a human genomic library constructed in the vector λFIX (Stratagene catalog # 944201) are plated and duplicate nitrocellulose replicas made. Two

30   oligonucleotides probes designed on the basis of nucleotides #1665-#1704 and #1837-#1876 of the sequence set forth in Figure 1 (SEQ ID NO:1) are synthesized on an automated DNA synthesizer. The sequence of these two oligonucleotides is indicated below:

        #1: CTATGAGTGTAAAGGGGGTTGCTTCTTCCCATTGGCTGAT

35   #2: GTGCCAACCCTCAAGTACCACTATGAGGGGATGAGTGTGG
These two oligonucleotide probes are radioactively labeled with

γ^{32}P-ATP and each is hybridized to one set of the duplicate nitrocellulose replicas in SHB at 65°C and washed with 1X SSC, 0.1% SDS at 65°C. Three recombinants which hybridize to both oligonucleotide probes are noted. All three positively hybridizing recombinants are plaque purified, bacteriophage plate stocks are prepared and bacteriophage DNA is isolated from each. The oligonucleotide hybridizing regions of one of these recombinants, designated HG111, is localized to a 1.2 kb Pst I/Xba I fragment. This fragment is subcloned into a plasmid vector (pGEM-3) and DNA sequence analysis is performed. HG111 was deposited with the American Type Culture Collection ATCC, 12301 Parklawn Drive, Rockville, Maryland USA (hereinafter the "ATCC") on June 16, 1992 under the requirements of the Budapest Treaty and designated as ATCC #75252. This subclone is designated pGEM-111. A portion of the DNA sequence of clone pGEM-111 is set forth in Figure 3 (SEQ ID NO:8/HUMAN BMP-9 SEQUENCE). This sequence encodes the entire mature region of human BMP-9 and a portion of the propeptide. It should be noted that this sequence consists of preliminary data. Particularly, the propeptide region is subject to further analysis and characterization. For example, nucleotides #1 through #3 (TGA) encode a translational stop which may be incorrect due to the preliminary nature of the sequence. It is predicted that additional sequences present in both pGEM-111 (the 1.2 kb PstI/XbaI fragment of HG111 subcloned into pGEM) and HG111 encode additional amino acids of the human BMP-9 propeptide region. Based on knowledge of other BMPs and other proteins within the TGF-β family, it is predicted that the precursor polypeptide would be cleaved at the multibasic sequence ARG-ARG-LYS-ARG (amino acids # -4 through # -1 of SEQ ID NO:9) in agreement with a proposed consensus proteolytic processing sequence ARG-X-X-ARG. Cleavage of the human BMP-9 precursor polypeptide at this location would generate a 110 amino acid mature peptide beginning with the amino acid SER at position #1 of SEQ ID NO:9 (encoded by nucleotides #124 through #126 of SEQ ID NO:8). The processing of human BMP-9 into the mature form is expected to involve dimerization and removal of the N-terminal region in a manner

analogous to the processing of the related protein TGF-β [L.E. Gentry et al., Mol. & Cell. Biol. 8:4162 (1988); R. Derynck et al., Nature 316:701 (1985)].

It is contemplated therefore that the mature active species of human BMP-9 comprises a homodimer of two polypeptide subunits, each subunit comprising amino acids #1 through #110 of SEQ ID NO:9, with a predicted molecular weight of 12,000 daltons. Further active species are contemplated comprising amino acids #8 through #110 thereby including the first conserved cysteine residue. As with other members of the BMP and TGF-β family of proteins, the carboxy-terminal portion of the human BMP-9 sequence exhibits greater sequence conservation than the amino-terminal portion. The percent amino acid identity of the human BMP-9 protein in the cysteine-rich C-terminal domain (amino acids #8 through #110) to the corresponding region of other human BMP proteins and other proteins within the TGF-β family is as follows: BMP-2, 52%; BMP-3, 40%; BMP-4, 52%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; murine BMP-9, 97%; Vg1, 50%; GDF-1, 44%; TGF-β1, 32%; TGF-β2, 32%; TGF-β3, 32%; inhibin β (B), 35%; and inhibin β (A), 41%. BMP-9 exhibits 80% homology to chick Dorsalin-1, a BMP-like protein cloned from embryonic chick.

EXAMPLE III

ROSEN MODIFIED SAMPATH-REDDI ASSAY

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. USA 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of the BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1 % TFA, and the resulting solution added to 20mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted

subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci. 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 11m glycolmethacrylate sections are stained with Von Kossa and acid fuschin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone. In a modified scoring method, three non-adjacent sections are evaluated from each implant and averaged. "+/-" indicates tentative identification of cartilage or bone; "+1" indicates >10% of each section being new cartilage or bone; "+2", >25%; "+3", >50%; "+4", ~75%; "+5", >80%. A "-" indicates that the implant is not recovered.

It is contemplated that the dose response nature of the BMP-9 containing samples of the matrix samples will demonstrate that the amount of bone and/or cartilage formed increases with the amount of BMP-9 in the sample. It is contemplated that the control samples will not result in any bone and/or cartilage formation.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing followed by autoradiography. The activity is correlated with the protein bands and pI. To estimate the purity of the protein in a particular fraction an extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and the protein is run on SDS

PAGE followed by silver staining or radioiodination and autoradiography.

EXAMPLE IV

EXPRESSION OF BMP-9

5   In order to produce murine, human or other mammalian BMP-9 proteins, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. The preferred

10   expression system for biologically active recombinant human BMP-9 is contemplated to be stably transformed mammalian cells.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Figure 1 (SEQ ID NO:1) or Figure 3 (SEQ ID NO:8), or other DNA sequences encoding BMP-9

15   proteins or other modified sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol. 2:161-170 (1982)], pJL3, pJL4 [Gough et al., EMBO J. 4:645-653 (1985)] and pMT2 CXM.

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) (Wong et al., Science 228:810-815 (1985)) differing

20   from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described (Kaufman, R.J., Proc. Natl. Acad. Sci. USA 82:689-693 (1985)) and include

25   the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site

30   (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 CXM is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC #67122. EcoRI digestion excises the cDNA insert present in

35   pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin

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resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga et al., Biotechnology 84:636 (1984)]. This removes bases 1075 to 1145 relative to the Hind III site near the

5   SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

5' PO-CATGGGCAGCTCGAG-3' (SEQ ID NO:5)

at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease Xho I. A derivative of pMT2CXM,

10   termed pMT23, contains recognition sites for the restriction endonucleases PstI, Eco RI, SalI and XhoI. Plasmid pMT2 CXM and pMT23 DNA may be prepared by conventional methods.

pEMC2b1 derived from pMT21 may also be suitable in practice of the invention. pMT21 is derived from pMT2 which is derived

15   from pMT2-VWF. As described above EcoRI digestion excises the cDNA insert present in pMT-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

20   pMT21 is derived from pMT2 through the following two modifications. First, 76 bp of the 5' untranslated region of the DHFR cDNA including a stretch of 19 G residues from G/C tailing for cDNA cloning is deleted. In this process, a XhoI site is inserted to obtain the following sequence immediately

25   upstream from DHFR: 5' -CTGCAGGCGAGCCTGAATTCCTCGAGCCATCATG-3'
                              PstI   Eco RI XhoI
(SEQ ID NO:6)

Second, a unique ClaI site is introduced by digestion with EcoRV and XbaI, treatment with Klenow fragment of DNA polymerase I, and

30   ligation to a ClaI linker (CATCGATG). This deletes a 250 bp segment from the adenovirus associated RNA (VAI) region but does not interfere with VAI RNA gene expression or function. pMT21 is digested with EcoRI and XhoI, and used to derive the vector pEMC2B1.

35   A portion of the EMCV leader is obtained from pMT2-ECAT1 [S.K. Jung et al., J. Virol 63:1651-1660 (1989)] by digestion with Eco RI and PstI, resulting in a 2752 bp fragment. This fragment is digested with TaqI yielding an Eco RI-TaqI fragment

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of 508 bp which is purified by electrophoresis on low melting
agarose gel. A 68 bp adapter and its complementary strand are
synthesized with a 5' TaqI protruding end and a 3' XhoI
protruding end which has the following sequence:

5   5'-<u>CGA</u>GGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTT
         TaqI

       GAAAAACACG<u>ATTGC</u>-3'
                 XhoI   (SEQ ID NO:7)

10   This sequence matches the EMC virus leader sequence from
       nucleotide 763 to 827. It also changes the ATG at position 10
       within the EMC virus leader to an ATT and is followed by a XhoI
       site. A three way ligation of the pMT21 Eco RI-XhoI fragment,
       the EMC virus EcoRI-TaqI fragment, and the 68 bp oligonucleotide

15   adapter TaqI-XhoI adapter resulting in the vector pEMC2β1.

       This vector contains the SV40 origin of replication and
       enhancer, the adenovirus major late promoter, a cDNA copy of the
       majority of the adenovirus tripartite leader sequence, a small
       hybrid intervening sequence, an SV40 polyadenylation signal and

20   the adenovirus VA I gene, DHFR and β-lactamase markers and an EMC
       sequence, in appropriate relationships to direct the high level
       expression of the desired cDNA in mammalian cells.

       The construction of vectors may involve modification of the
       BMP-9 DNA sequences. For instance, BMP-9 cDNA can be modified

25   by removing the non-coding nucleotides on the 5' and 3' ends of
       the coding region. The deleted non-coding nucleotides may or may
       not be replaced by other sequences known to be beneficial for
       expression. These vectors are transformed into appropriate host
       cells for expression of BMP-9 proteins. One skilled in the art

30   can manipulate the sequences of Figure 1 or Figure 3 (SEQ ID
       NOS:1 and 8) by eliminating or replacing the mammalian regulatory
       sequences flanking the coding sequence with bacterial sequences
       to create bacterial vectors for intracellular or extracellular
       expression by bacterial cells. For example, the coding sequences

35   could be further manipulated (e.g., ligated to other known
       linkers or modified by deleting non-coding sequences therefrom
       or altering nucleotides therein by other known techniques). The
       modified BMP-9 coding sequence could then be inserted into a

known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and a BMP-9 protein expressed thereby. For

5   a strategy for producing extracellular expression of BMP-9 proteins in bacterial cells, see e.g., European Patent Application No. EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See e.g., procedures described in published

10 European Patent Application No. 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See e.g., procedures described in published PCT

15 Publication No. WO86/00639 and European Patent Application No. EPA 123,289].

A method for producing high levels of a BMP-9 protein of the invention in mammalian cells may involve the construction of cells containing multiple copies of the heterologous BMP-9 gene.

20 The heterologous gene is linked to an amplifiable marker, e.g., the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol. 159:601-629

25 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a BMP-9 of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression

30 plasmid pAdA26SV(A)3 [Kaufman and Sharp, Mol. Cell. Biol. 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by various methods including calcium phosphate coprecipitation and transfection, electroporation or protoplast fusion. DHFR expressing transformants are selected for growth

35 in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (e.g., sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as

described in Kaufman et al., Mol Cell Biol. 5:1750 (1983).
Transformants are cloned, and biologically active BMP-9
expression is monitored by the Rosen-modified Sampath - Reddi rat
bone formation assay described above in Example III. BMP-9
5   expression should increase with increasing levels of MTX
resistance. BMP-9 polypeptides are characterized using standard
techniques known in the art such as pulse labeling with [35S]
methionine or cysteine and polyacrylamide gel electrophoresis.
Similar procedures can be followed to produce other related BMP-9
10   proteins.

A.   BMP-9 VECTOR CONSTRUCTION

       In order to produce human BMP-9 proteins of the invention
DNA sequences encoding the mature region of the human BMP-9
protein may be joined to DNA sequences encoding the propeptide
15   region of the murine BMP-9 protein. This murine/human hybrid DNA
sequence is inserted into an appropriate expression vector and
introduced into mammalian cells or other preferred eukaryotic or
prokaryotic hosts by conventional genetic engineering techniques.
The construction of this murine/human BMP-9 containing expression
20   plasmid is described below.

       A derivative of the human BMP-9 sequence (SEQ ID NO:8)
comprising the nucleotide sequence from nucleotide #105 to #470
is specifically amplified. The following oligonucleotides are
utilized as primers to allow the amplification of nucleotides
25   #105 to #470 of the human BMP-9 sequence (SEQ ID NO:8) from clone
pGEM-111 described above.

       #3 ATCGGGCCCCTTTTAGCCAGGCGGAAAAGGAG
       #4 AGCGAATTCCCCGCAGGCAGATACTACCTG

This procedure generates the insertion of the nucleotide sequence
30   ATCGGGCCCCT immediately preceding nucleotide #105 and the
insertion of the nucleotide sequence GAATTCGCT immediately
following nucleotide #470. The addition of these sequences
results in the creation of an Apa I and EcoR I restriction
endonuclease site at the respective ends of the specifically
35   amplified DNA fragment. The resulting 374 bp Apa I/EcoR I
fragment is subcloned into the plasmid vector pGEM-7Zf(+)

(Promega catalog# p2251) which has been digested with Apa I and
EcoR I. The resulting clone is designated phBMP9mex-1.

The following oligonucleotides are designed on the basis of
murine BMP-9 sequences (SEQ ID NO:1) and are modified to
5   facilitate the construction of the murine/human expression
plasmid referred to above:

#5

GATTCCGTCGACCACCATGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC

#6 CCACAGCTGTGTATCCATCTAGACCAGGCCCCAGGGGACATGGTGGTCGACG

10   These oligonucleotides contain complimentary sequences which upon
addition to each other facilitate the annealing (base pairing)
of the two individual sequences, resulting in the formation of
a double stranded synthetic DNA linker (designated LINK-1) in a
manner indicated below:

15   1   5   10   20   30   40   50   60
        | | | | | | | |
    #5GATTCCGTCGACCACCATGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC
            GCAGCTGGTGGTACAGGGGACCCCGGACCAGATCTACCTATGTGTCGACACC #6

This DNA linker (LINK-1) contains recognition sequences of
20   restriction endonucleases needed to facilitate subsequent
manipulations required to construct the murine/human expression
plasmid, as well as sequences required for maximal expression of
heterologous sequences in mammalian cell expression systems.
More specifically (referring to the sequence numbering of
25   oligonucleotide #5/LINK-1): nucleotides #1-#11 comprise
recognition sequences for the restriction endonucleases BamH I
and Sal I, nucleotides #11-#15 allow for maximal expression of
heterologous sequences in mammalian cell expression systems,
nucleotides #16-#31 correspond to nucleotides #610-#625 of the
30   murine BMP-9 sequence (SEQ ID NO:1), nucleotides #32-#33 are
inserted to facilitate efficient restriction digestion of two
adjacent restriction endonuclease sites (EcoO109 I and Xba I),
nucleotides #34-#60 correspond to nucleotides #1515-#1541 of the
murine BMP-9 sequence (SEQ ID NO:1) except that nucleotide #58
35   of synthetic oligonucleotide #5 is a G rather than the A which
appears at position #1539 of SEQ ID NO:1 (This nucleotide
conversion results in the creation of an Apa I restriction
endonuclease recognition sequence, without altering the amino

acid sequence it is intended to encode, to facilitate further manipulations of the murine/human hybrid expression plasmid. LINK-1 (the double stranded product of the annealing of oligonucleotides #5 and #6) is subcloned into the plasmid vector

5   pGEM-7Zf(+) which has been digested with the restriction endonucleases Apa I and BamH I. This results in a plasmid in which the sequences normally present between the Apa I and BamH I sites of the pGEM-7Zf(+) plasmid polylinker are replaced with the sequences of LINK-1 described above. The resulting plasmid

10   clone is designated pBMP-9link.

        pBMP-9link is digested with the restriction endonucleases BamH I and Xba I resulting in the removal nucleotides #1-#34 of LINK-1 (refer to the numbering of oligo #5). Clone ML14a, which contains an insert comprising the sequence set forth in SEQ ID

15   NO:1, is also digested with the restriction endonucleases BamH I and Xba I resulting in the removal of sequences comprising nucleotides #1-#1515 of SEQ ID NO:1 (murine BMP-9). This BamH I/Xba I fragment of mouse BMP-9 is isolated from the remainder of the ML14a plasmid clone and subcloned into the BamH I/Xba I

20   sites generated by the removal of the synthetic linker sequences described above. The resulting clone is designated p302.

        The p302 clone is digested with the restriction endonuclease EcoO109 I resulting in the excision of nucleotides corresponding to nucleotides #621-#1515 of the murine BMP-9 sequence (SEQ ID

25   NO:1) and nucleotides #35-#59 of LINK-1 (refer to numbering of oligonucleotide #5). It should be noted that the Apa I restriction site created in LINK-1 by the A to G conversion described above is a subset of the recognition sequence of EcoO109 I, therefore digestion of p302 with EcoO109 I cleaves at

30   the Apa I site as well as the naturally occurring murine EcoO109 I (location #619-#625 of SEQ ID NO:1) resulting in the excision of a 920 bp EcoO109 I/EcoO109 I (Apa I) fragment comprising the sequences described above. This 920 EcoO109 I/EcoO109 I (Apa I) fragment is isolated from the remainder of the p302 plasmid clone

35   and subcloned into clone pBMP-9link which has been similarly digested with EcoO109 I. It should be noted that the nucleotides GG (#32-#33 of oligonucleotide #5) originally designed to

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facilitate a more complete digestion of the two adjacent restriction sites EcoO109 I and Xba I of LINK-1, which is now a part of pBMP-9link (described above), results in the creation of Dcm methylation recognition sequence. The restriction nuclease EcoO109 I is sensitive to Dcm methylation and therefore cleavage of this sequence (nucleotides #25-#31 of oligonucleotide #5/LINK-1) by the restriction endonuclease EcoO109 I is prevented at this site. Therefore the plasmid clone pBMP-9link is cleaved at the Apa I site but not at the EcoO109 I site upon digestion with the restriction endonuclease EcoO109 I as described above, preventing the intended removal of the sequences between the EcoO109 I and Xba I site of LINK-1 (#32-#55 defined by the numbering of oligonucleotide #5). This results in the insertion of the 920 bp EcoO109 I/Apa I fragment at the EcoO109 I (Apa I) site of pBMP-9link. The resulting clone is designated p318.

Clone p318 is digested with the restriction endonucleases Sal I and Apa I, resulting in the excision of sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location), nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1), and nucleotides #35-#60 of LINK-1 (refer to oligo #5 for location). The resulting 972 bp Sal I/Apa I fragment described above is isolated from the remainder of the p318 plasmid clone and will be utilized in subsequent manipulations.

The clone phBMP9mex-1 (described above), which contains DNA sequences which encode the entire mature region and portions of the propeptide of the human BMP-9 protein, is digested with the restriction endonucleases Apa I and EcoR I. This results in the excision of a 374 bp fragment comprising nucleotides #105-#470 of the human BMP-9 sequence (SEQ ID NO:8) and the additional nucleotides of oligonucleotide primers #3 and #4 which contain the recognition sequences for the restriction endonucleases Apa I and EcoR I. This 374 bp Apa I/EcoR I fragment is combined with the 972 bp Sal I/Apa I fragment from p138 (isolation described above) and ligated to the mammalian cell expression plasmid pED6 (a derivative of pEMC2β1) which has been digested with Sal I and EcoR I. The resulting clone is designated p324.

The clone ML14a (murine BMP-9) is digested with EcoO109 I

24

and Xba I to generate a fragment comprising nucleotides #621-#1515 of SEQ ID NO:1.

The following oligonucleotides are synthesized on an automated DNA synthesizer and combined such that their complimentary sequences can base pair (anneal) with each other to generate a double stranded synthetic DNA linker designated LINK-2:

    #7 TCGACCACCATGTCCCCTGG
    #8 GCCCCAGGGGACATGGTGG

This double stranded synthetic DNA linker (LINK-2) anneals in such a way that it generates single stranded ends which are compatible to DNA fragments digested with Sal I (one end) or EcoO109 I (the other end) as indicated below:

    #7 TCGACCACCATGTCCCCTGG
          GGTGGTACAGGGGACCCCG #8

This LINK-2 synthetic DNA linker is ligated to the 895 bp EcoO109 I/Xba I fragment comprising nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) described above. This results in a 915 bp Sal I/Xba I fragment.

The clone p324 is digested with Sal I/Xba I to remove sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location) and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1). The sequences comprising nucleotides #35-#60 of LINK-1 (refer to oligo #5 for location) and the sequences comprising the 374 bp Apa I/EcoR I fragment (human BMP-9 sequences) derived from phBMP9mex-1 remain attached to the pED6 backbone. The 915 bp Sal I/Xba I fragment comprising LINK-2 sequences and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) is ligated into the p324 clone from which the Sal I to Xba I sequences described above have been removed.

The resulting plasmid is designated BMP-9 fusion and comprises LINK-2, nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1), nucleotides #35-#59 of LINK-1 (refer to the numbering of oligonucleotide #5), and the 374 bp Apa I/EcoR I fragment (human BMP-9) derived from clone pBMP9mex-1 (described above) inserted between the Sal I and EcoR I sites of the mammalian cell expression vector pED6.

B.   EXPRESSION

BMP-9 fusion is transfected into CHO cells using standard techniques known to those having ordinary skill in the art to create stable cell lines capable of expressing human BMP-9
5   protein.   The cell lines are cultured under suitable culture conditions and the BMP-9 protein is isolated and purified from the culture medium.

In one embodiment, cells are grown in R1 medium based on a 50:50 mix of F12 and DME plus extra non-essential amino acids
10   plus extra biotin and B12 and 10% fetal bovine serum (FBS) and 0.2 çM methotrexate (MTX).   Cells are grown up and expanded into roller bottles in this medium using confluent roller bottles. The serum containing growth medium is discarded, the rollers are rinsed with PBS-CMF, and a serum free production medium is added
15   containing additional amino acids plus insulin (5 çg/ml), putrescine (12.9 çM), hydrocortisone (0.2 çM), selenium (29 nM), and PVA (0.6 g/L).   Dextran sulfate is used in this CM (at 100 çg/ml).   Conditioned medium (CM) is collected at 24 hours and the rollers are refed with fresh serum free medium.   Four sequential
20   24 hour harvest can be collected.   Conditioned medium is clarified (floating cells in the CM are removed) for purification by passing the CM through a 5 ç (pass Profile) pore size filter and a 0.22 ç (millipore Duropore) pore size filter.

EXAMPLE V

25   **BIOLOGICAL ACTIVITY OF EXPRESSED BMP-9**

To measure the biological activity of the expressed BMP-9 proteins obtained in Example IV above, the proteins are recovered from the cell culture and purified by isolating the BMP-9 proteins from other proteinaceous materials with which they are
30   co-produced as well as from other contaminants.   The purified protein may be assayed in accordance with the rat bone formation assay described in Example III.

Purification is carried out using standard techniques known to those skilled in the art.   It is contemplated, as with other
35   BMP proteins, that purification may include the use of Heparin sepharose.

26

In one embodiment, 40 liters of the conditioned media from Example IV-B is titrated to pH 6.9 with concentrated sodium phosphate pH 6.0, and loaded onto Cellufine Sulfate, previously equilibrated with 50 mM sodium phosphate, pH 6.9. The resin is washed with 50 mM sodium phosphate, 0.5 M NaCl, followed by 50 mM sodium phosphate, 0.5 M NaCl, 0.5 M Arg, pH 6.9. BMP-9 is found in the wash as well as the elution, with a lesser amount of contaminants in the elution pool. Cellufine sulfate pools are concentrated and directly loaded onto RP-HPLC for final purification. Each concentrated pool is titrated to pH 3.8 with dilute TFA and loaded onto a 0.46 X 25 cm C$_4$ reverse phase column running a linear gradient from 30% A (0.1% TFA/H$_2$O) to 55% B (0.1% TFA/90% Acetonitrile) over 100 minutes. BMP-9 monomer is separated by baseline resolution from BMP-9 dimer. The identity of monomer and dimer pools are confirmed by N-terminal sequencing. Although heterogeneity in the N-terminus is expected sequencing reveals a predominant species Ser-Ala-Gly-Ala beginning with amino acid #1 of SEQ ID NO:9.

Protein analysis is conducted using standard techniques such as SDS-PAGE acrylamide [U.K. Laemmli, Nature 227:680 (1970)] stained with silver [R.R. Oakley et al., Anal. Biochem. 105:361 (1980)] and by immunoblot [H. Towbin et al., Proc. Natl. Acad. Sci. USA 76:4350 (1979)]. BMP-9 is efficiently expressed in CHO cells as a 14kDa nonglycosylated protein when analyzed under reducing conditions. BMP-9 is efficiently secreted within 4 hours of its synthesis.

EXAMPLE VI

A. W-20 BIOASSAY

Use of the W-20 bone marrow stromal cells as an indicator cell line is based upon the conversion of these cells to osteoblast-like cells after treatment with BMP-2 [R. S. Thies et al., "Bone Morphogenetic Protein alters W-20 stromal cell differentiation in vitro", Journal of Bone and Mineral Research 5(2):305 (1990); and R. S. Thies et al., "Recombinant Human Bone Morphogenetic Protein 2 Induces Osteoblastic Differentiation in W-20-17 Stromal Cells", Endocrinology, in press (1992)].

Specifically, W-20 cells are a clonal bone marrow stromal cell line derived from adult mice by researchers in the laboratory of Dr. D. Nathan, Children's Hospital, Boston, MA. BMP-2 treatment of W-20 cells results in (1) increased alkaline phosphatase production, (2) induction of PTH stimulated cAMP, and (3) induction of osteocalcin synthesis by the cells. While (1) and (2) represent characteristics associated with the osteoblast phenotype, the ability to synthesize osteocalcin is a phenotypic property only displayed by mature osteoblasts. Furthermore, to date we have observed conversion of W-20 stromal cells to osteoblast-like cells only upon treatment with BMPs. In this manner, the _in vitro_ activities displayed by BMP treated W-20 cells correlate with the _in vivo_ bone forming activity known for BMPs.

Below two _in vitro_ assays useful in comparison of BMP activities of novel osteoinductive molecules are described.

B.   W-20 ALKALINE PHOSPHATASE ASSAY PROTOCOL

W-20 cells are plated into 96 well tissue culture plates at a density of 10,000 cells per well in 200 μl of media (DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 100 U/ml + 100 μg/ml streptomycin. The cells are allowed to attach overnight in a 95% air, 5% CO_2 incubator at 37°C.

The 200 μl of media is removed from each well with a multichannel pipettor and replaced with an equal volume of test sample delivered in DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 1% penicillin-streptomycin. Test substances are assayed in triplicate.

The test samples and standards are allowed a 24 hour incubation period with the W-20 indicator cells. After the 24 hours, plates are removed from the 37°C incubator and the test media are removed from the cells.

The W-20 cell layers are washed 3 times with 200 μl per well of calcium/magnesium free phosphate buffered saline and these washes are discarded.

50 μl of glass distilled water is added to each well and the assay plates are then placed on a dry ice/ethanol bath for quick

freezing. Once frozen, the assay plates are removed from the dry ice/ethanol bath and thawed at 37°C. This step is repeated 2 more times for a total of 3 freeze-thaw procedures. Once complete, the membrane bound alkaline phosphatase is available for measurement.

5

50 μl of assay mix (50 mM glycine, 0.05% Triton X-100, 4 mM MgCl$_2$, 5 mM p-nitrophenol phosphate, pH = 10.3) is added to each assay well and the assay plates are then incubated for 30 minutes at 37°C in a shaking waterbath at 60 oscillations per minute.

10

At the end of the 30 minute incubation, the reaction is stopped by adding 100 μl of 0.2 N NaOH to each well and placing the assay plates on ice.

The spectrophotometric absorbance for each well is read at a wavelength of 405 nanometers. These values are then compared

15

to known standards to give an estimate of the alkaline phosphatase activity in each sample. For example, using known amounts of p-nitrophenol phosphate, absorbance values are generated. This is shown in Table I.

20

Table I
Absorbance Values for Known Standards
of P-Nitrophenol Phosphate

P-nitrophenol phosphate umoles	Mean absorbance (405 nm)
0.000	0
0.006	0.261 +/- .024
0.012	0.521 +/- .031
0.018	0.797 +/- .063
0.024	1.074 +/- .061
0.030	1.305 +/- .083

25

30

Absorbance values for known amounts of BMP-2 can be determined and converted to μmoles of p-nitrophenol phosphate cleaved per unit time as shown in Table II.

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--------------------------------------------------------------
```
                                  Table II
                  Alkaline Phosphatase Values for W-20 Cells
                            Treating with BMP-2

BMP-2 concentration ng/ml	Absorbance Reading 405 nmeters	umoles substrate per hour
0	0.645	0.024
1.56	0.696	0.026
3.12	0.765	0.029
6.25	0.923	0.036
12.50	1.121	0.044
25.0	1.457	0.058
50.0	1.662	0.067
100.0	1.977	0.080

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These values are then used to compare the activities of known amounts of BMP-9 to BMP-2.

C.   **OSTEOCALCIN RIA PROTOCOL**

W-20 cells are plated at 10^6 cells per well in 24 well multiwell tissue culture dishes in 2 mls of DME containing 10% heat inactivated fetal calf serum, 2 mM glutamine. The cells are allowed to attach overnight in an atmosphere of 95% air 5% CO_2 at 37°C.

The next day the medium is changed to DME containing 10% fetal calf serum, 2 mM glutamine and the test substance in a total volume of 2 ml. Each test substance is administered to triplicate wells. The test substances are incubated with the W-20 cells for a total of 96 hours with replacement at 48 hours by the same test medias.

At the end of 96 hours, 50 μl of the test media is removed from each well and assayed for osteocalcin production using a radioimmunoassay for mouse osteocalcin. The details of the assay are described in the kit manufactured by Biomedical Technologies Inc., 378 Page Street, Stoughton, MA 02072. Reagents for the assay are found as product numbers BT-431 (mouse osteocalcin standard), BT-432 (Goat anti-mouse Osteocalcin), BT-431R (iodinated mouse osteocalcin), BT-415 (normal goat serum) and BT-414 (donkey anti goat IgG). The RIA for osteocalcin synthesized by W-20 cells in response to BMP treatment is carried out as

described in the protocol provided by the manufacturer.

The values obtained for the test samples are compared to values for known standards of mouse osteocalcin and to the amount of osteocalcin produced by W-20 cells in response to challenge with known amounts of BMP-2.

```
-----------------------------------------------------------------
                            Table III
            · Osteocalcin Synthesis by W-20 Cells

        BMP-2 Concentration ng/ml   Osteocalcin Synthesis ng/well

                0                           0.8
                2                           0.9
                4                           0.8
                8                           2.2
               16          .                2.7
               31                           3.2
               62                           5.1
              125                           6.5
              250                           8.2
              500                           9.4
             1000                          10.0

-----------------------------------------------------------------
```

EXAMPLE VII

ARTICULAR CARTILAGE ASSAY

The effect of BMP-9 on articular cartilage proteoglycan and DNA synthesis is assayed to determine if BMP-9 is involved in the regulation of metabolism of differentiated articular cartilage.

Articular cartilage explants from calf carpal joints are maintained in DMEM with 50 μg/ml ascorbate, 4 mM glutamine and antibiotics for 3 days. Cytokines (rhBMP-2, rhBMP-4, rhBMP-6 and rhBMP-9, IGF-1, bFGF (1-1000 ng/ml), and TGFβ (1-100 ng/ml)) are added to the medium and culture is continued for 3 more days. Medium is changed daily. Twenty-four hours prior to harvest, explants are pulsed with 50 μCi/ml ^{35}SO$_4$ or 25 μCi/ml ^{3}H-thymidine. Explants are solubilized and separation of free isotope is performed by gel chromatography. Total DNA of each explant is measured by a spectrophotometric assay. BMP-9 stimulates proteoglycan synthesis above control levels at a dose of 10 ng/ml (p<0.05).

BMP-4, BMP-6, BMP-9 and TGFβ are significantly more active in stimulating proteoglycan synthesis at 100 ng/ml. At the

31

highest doses of cytokine tested (1 μg/ml), proteoglycan synthesis by explants exposed to all cytokines are significantly greater (p<0.05) than that by control explants. Sulfate incorporation results are set forth in Figure 4.

5   Recombinant human BMP-9 stimulates alkaline phosphatase activity in the osteoprogenitor cell line, W-20-17, in a dose responsive manner with an ED_{50} of 4 ng/ml. In vivo, high doses are rhBMP-9 induce ectopic bone formation, with 25 μg/implant of rhBMP-9 inducing cartilage and bone tissue after 10 days of

10   implantation.

EXAMPLE VIII

STIMULATION OF LIVER CELLS

     It is contemplated that BMP-9 may be used in liver repair or regeneration. Through the use of whole embryo sections or

15   whole mount techniques, expression of mRNA in multiple tissue is screened simultaneously. In the 11.5 dpc mouse embryo, BMP-9 mRNA localizes exclusively to the developing liver. It is contemplated that BMP-9, like all other BMPs studied to date, acts as a local regulator of cell growth and differentiation,

20   therefore this very specific expression pattern suggests liver as a BMP-9 target tissue.

     BMP-9 responsiveness in parenchymal liver cells is tested by screening four liver cell lines for their ability to bind iodinated, CHO-derived BMP-9. The four liver cell lines, HepG2

25   (ATCC HB8065), NMuli (ATCC CRL1638), Chang and NCTC1469 (ATCC CCL9.1), all specifically bind ^{125}I-BMP-9 to some extent, with HepG2 and NCTC1469 cell lines exhibiting the highest degree of binding. Specific binding of BMP-9 to HepG2 cells is carried out by incubating HepG2 cells grown to confluence in Dulbecco's

30   Modified Eagle's Medium (DME) containing 10% heat-inactivated fetal calf serum (FCS) on gelatinized 6 well plates with 2 ng/ml ^{125}I-BMP-9 and increasing concentrations of unlabelled BMP-9 in binding buffer (136.9 mM NaCl, 5.37 mM KCl, 1.26 mM $CaCl_2$, 0.64 mM $MgSO_4$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.49 mM $MgCl_2$, 25 mM

35   HEPES and 0.5% BSA, pH 7.4) for 20 hours at 4°C to achieve binding equilibrium. This incubation follows a one hour

preincubation at 37°C in binding buffer alone. For crosslinking experiments, the cells were incubated with 500 μM disuccinimidyl suberate for 20 minutes at 4°C following binding. Cell extracts were analyzed on SDS-PAGE. As shown in Figure 5, HepG2 cells

5   expressed abundant high affinity receptors for BMP-9. Scatchard analysis of these binding data resulted in a curvilinear plot, with approximately 10,000 high affinity receptors per cell. These receptors exhibited a K_d of 0.3 nM. The curvilinear nature of the Scatchard plot indicates negative cooperativity among BMP-

10   9 receptors or that HepG2 cells express at least two populations of BMP-9 receptors with different affinities. Crosslinking analysis on HepG2 cells with ^{125}I-BMP-9 yields two binding proteins of apparent molecular weights of 54 and 80 kD. Crosslinked ligand/receptor complexes were observed at 78 and 100

15   kD under nonreducing conditions, and 67 and 94 kD under reducing conditions. Subtracting the molecular weight of the BMP-9 dimer and monomer, respectively, it is estimated that these BMP-9 receptor proteins have molecular weights of approximately 54 and 80 kD. The K_d of the high affinity binding sites for BMP-9 is

20   estimated to be approximately 270 pM for HepG2 cells. To test the binding specificity of the receptors for BMP-9, HepG2 cells were incubated with ^{125}I-BMP-9 and a 250-fold excess of different unlabeled ligands. The BMP-9 receptors expressed on HepG2 cells show only limited crossreactivity with BMPs 2 and 4, and no

25   crossreactivity with BMPs 3, 5, 6, 7, 12 and 2/6, or with TGF-ß1 or TGF-ß2.

As a first indication of BMP-9 effects on confluent, serum starved HepG2 cells, cell proliferation is examined as determined by ^{3}H-thymidine incorporation and cell counting. HepG2 cells are

30   plated at 10^6 cells/well in 96 well plates and cultured for 48 hours in DME/0.1% FCS to synchronize the cell cycle are treated for 24 hours with or without BMP-9 in the presence of 0.1% FCS. In ^{3}H-thymidine incorporation assays, ^{3}H-thymidine was included during the last 4 hours of treatment and cellular DNA was

35   collected with a 96 well plate cell harvester. Proliferation was assayed by quantifying ethanol-precipitable ^{3}H-thymidine incorporation by liquid scintillation counting. For cell

counting assays, cells were trypsinized and counted with a
hemacytometer.   Primary rat hepatocytes isolated from male
Fischer 344 rats (Charles River, Wilmington, MA) by collagenase
digestion as perviously described [Michalopoulos et al., Cancer
5   Res. 42:4673-4682 (1982)] are plated on collagen-coated plates
at subconfluence (5,000-10,000 cells/cm^2) in serum-free media as
described in Michalopoulos et al., Cancer Res. 42:4673-4682
(1982) and treated with or without rhBMP-9 for 36 hours. ^{3}H-
thymidine was included throughout the treatment period and
10   incorporated ^{3}H-thymidine was quantified as described by Anscher
et al., New England J. Med. 328:1592-1598 (1993).   BMP-9
stimulates ^{3}H-thymidine   incorporation   in   HepG2   cells
approximately five fold.   This effect is confirmed by a
stimulatory effect of BMP-9 in cell counting experiments.   As
15   shown in Figure 6, BMP-9 stimulated ^{3}H-thymidine incorporation in
HepG2 cells in a dose-responsive manner. The ED_{50} for this effect
was estimated at 10 ng/ml BMP-9.   This ED_{50} value is consistent
with the estimated binding affinity (K_d = 0.3 nM = 8 ng/ml),
suggesting that this biological effect is mediated by the
20   described BMP-9 receptors.

To determine if this proliferative effect of BMP-9 was
unique to the HepG2 liver tumor cell line, primary rat
hepatocytes were tested for effects of BMP-9 on ^{3}H-thymidine
incorporated as shown in Figure 7. BMP-9 stimulated ^{3}h-thymidine
25   incorporation in primary hepatocytes, although not as markedly
as EGF.   This stimulatory effect is cell density-dependent in
primary rat hepatocytes.   While subconfluent cells exhibited a
stimulation in response to BMP-9, confluent primary hepatocytes
did not. As indicatedin Figure 7, in contrast to rhBMP-9, TGF-β1
30   was inhibitory, not stimulatory on primary rat hepatocytes.

The foregoing descriptions detail presently preferred
embodiments of the present invention. Numerous modifications and
variations in practice thereof are expected to occur to those
skilled in the art upon consideration of these descriptions.
35   Those modifications and variations are believed to be encompassed
within the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Rosen, Vicki A.
                  Wozney, John M.
                      Celeste, Anthony J.

    (ii) TITLE OF INVENTION: BMP-9 COMPOSITIONS

    (iii) NUMBER OF SEQUENCES: 9

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        (A) ADDRESSEE: Genetics Institute, Inc.
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        (C) CITY: Cambridge
        (D) STATE: MA
        (E) COUNTRY: US
        (F) ZIP: 02140

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER: US
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: Kapinos, Ellen J.
        (B) REGISTRATION NUMBER: 32,245
        (C) REFERENCE/DOCKET NUMBER: GI 5186C-PCT

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (617) 876-1210
        (B) TELEFAX: (617) 876-5851

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 2447 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Mus musculus
        (B) STRAIN: C57B46xCBA
        (F) TISSUE TYPE: liver

    (vii) IMMEDIATE SOURCE:
        (A) LIBRARY: Mouse liver cDNA
        (B) CLONE: ML14A

    (viii) POSITION IN GENOME:
        (C) UNITS: bp

35

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        (ix) FEATURE:
             (A) NAME/KEY: mat_peptide
             (B) LOCATION: 1564..1893

  5     (ix) FEATURE:
             (A) NAME/KEY: CDS
             (B) LOCATION: 610..1896

        (ix) FEATURE:
             (A) NAME/KEY: mRNA
             (B) LOCATION: 1..2447


 10     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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     ATGGCTATAC TTAGATTTAT GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG     180

     CCTAGGAGAT TTGTTGATCC AATAAATATG ATTAGGGAAA CAATTATTAG GGTTCATGTT     240

 15  CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTTGATAA TAAGTTTAAC TAGTCAGTGT      300

     TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGGAATAG GATTGAAGGA     360

     AATATAATGA TGGCTACAAC GATTGGGAAT CCTATTATTG TTGGGGTAAT GAATGAGGCA     420

     AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA     480

     TTGGTGAGTA GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA     540

 20  CCTGATGTTA GAAGGAGGGC TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG     600

     GCCACCCGG ATG TCC CCT GGG GCC TTC CGG GTG GCC CTG CTC CCG CTG       648
               Met Ser Pro Gly Ala Phe Arg Val Ala Leu Leu Pro Leu
               -318         -315                 -310

     TTC CTG CTG GTC TGT GTC ACA CAG CAG AAG CCG CTG CAG AAC TGG GAA     696
 25  Phe Leu Leu Val Cys Val Thr Gln Gln Lys Pro Leu Gln Asn Trp Glu
     -305                 -300                 -295                 -290

     CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC CTG GGA TTG TCT GGA     744
     Gln Ala Ser Pro Gly Glu Asn Ala His Ser Ser Leu Gly Leu Ser Gly
                 -285                 -280                 -275

 30  GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG GAG AAC ATG     792
     Ala Gly Glu Glu Gly Val Phe Asp Leu Gln Met Phe Leu Glu Asn Met
                 -270                 -265                 -260

     AAG GTG GAT TTC CTA CGC AGC CTT AAC CTC AGC GGC ATT CCC TCC CAG     840
     Lys Val Asp Phe Leu Arg Ser Leu Asn Leu Ser Gly Ile Pro Ser Gln
 35              -255                 -250                 -245

     GAC AAA ACC AGA GCG GAG CCA CCC CAG TAC ATG ATC GAC TTG TAC AAC     888
     Asp Lys Thr Arg Ala Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn
                 -240                 -235                 -230

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 40  Arg Tyr Thr Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg
     -225                 -220                 -215                 -210

     AGC TTC AGC GTG GAA GAT GCT ATA TCG ACA GCT GCC ACG GAG GAC TTC     984
     Ser Phe Ser Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe
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         Pro Phe Gln Lys His Ile Leu Ile Phe Asn Ile Ser Ile Pro Arg His
                    -190                -185                -180

  5      GAG CAG ATC ACC AGG GCT GAG CTC CGA CTC TAT GTC TCC TGC CAA AAT    1080
         Glu Gln Ile Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn
                    -175                -170                -165

         GAT GTG GAC TCC ACT CAT GGG CTG GAA GGA AGC ATG GTC GTT TAT GAT    1128
         Asp Val Asp Ser Thr His Gly Leu Glu Gly Ser Met Val Val Tyr Asp
                    -160                -155                -150

  10     GTT CTG GAG GAC AGT GAG ACT TGG GAC CAG GCC ACG GGG ACC AAG ACC    1176
         Val Leu Glu Asp Ser Glu Thr Trp Asp Gln Ala Thr Gly Thr Lys Thr
                    -145                -140                -135                -130

         TTC TTG GTA TCC CAG GAC ATT CGG GAC GAA GGA TGG GAG ACT TTA GAA    1224
         Phe Leu Val Ser Gln Asp Ile Arg Asp Glu Gly Trp Glu Thr Leu Glu
  15                -125                -120                -115

         GTA TCG AGT GCC GTG AAG CGG TGG GTC AGG GCA GAC TCC ACA ACA AAC    1272
         Val Ser Ser Ala Val Lys Arg Trp Val Arg Ala Asp Ser Thr Thr Asn
                    -110                -105                -100

  20     AAA AAT AAG CTC GAG GTG ACA GTG CAG AGC CAC AGG GAG AGC TGT GAC    1320
         Lys Asn Lys Leu Glu Val Thr Val Gln Ser His Arg Glu Ser Cys Asp
                    -95                 -90                 -85

         ACA CTG GAC ATC AGT GTC CCT CCA GGT TCC AAA AAC CTG CCC TTC TTT    1368
         Thr Leu Asp Ile Ser Val Pro Pro Gly Ser Lys Asn Leu Pro Phe Phe
                    -80                 -75                 -70

  25     GTT GTC TTC TCC AAT GAC CGC AGC AAT GGG ACC AAG GAG ACC AGA CTG    1416
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         Thr Ala Lys Asn Ala Tyr Gln Val Ala Gly Glu Ser Gln Glu Glu Glu
                    -30                 -25                 -20

         GGT CTA GAT GGA TAC ACA GCT GTG GGA CCA CTT TTA GCT AGA AGG AAG    1560
  35     Gly Leu Asp Gly Tyr Thr Ala Val Gly Pro Leu Leu Ala Arg Arg Lys
                    -15                 -10                 -5

         AGG AGC ACC GGA GCC AGC AGC CAC TGC CAG AAG ACT TCT CTC AGG GTG    1608
         Arg Ser Thr Gly Ala Ser Ser His Cys Gln Lys Thr Ser Leu Arg Val
                     1                   5                   10                  15

  40     AAC TTT GAG GAC ATC GGC TGG GAC AGC TGG ATC ATT GCA CCC AAG GAA    1656
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                            20                  25                  30

         TAT GAC GCC TAT GAG TGT AAA GGG GGT TGC TTC TTC CCA TTG GCT GAT    1704
         Tyr Asp Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp
  45                     35                  40                  45

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         Asp Val Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu
                        50                  55                  60

         GAG TTC CCC ACA AAG GTG GGC AAA GCC TGC TGC GTT CCC ACC AAA CTG    1800
  50     Glu Phe Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu
                        65                  70                  75
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37

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Lys Tyr His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
                100   105   110

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TGCGCATGGT ATGCCTAAGT TGATCAGAAA CCATCCTTGA GAAGAAAAGG AGTTAGTTGC   2023

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TGTCCTCAGG GAGAACAGCA TTGCTGTTCC TGTGCCTCAA GCTCCCAGCT GACTCTCCTG   2323

TGGCTCATAG GACTGAATGG GGTGAGGAAG AGCCTGATGC CCTCTGGCAA TCAGAGCCCG   2383

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AGAG   2447

(2) INFORMATION FOR SEQ ID NO:2:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 428 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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              -250   -245   -240

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Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser
          -220   -215   -210

Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe Pro Phe Gln
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-190   -185   -180   -175

38

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        -30                   -25                   -20                   -15

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                  5                   10                   15

 25     Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Asp Ala
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        Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr
          35                   40                   45                   50

        Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu Glu Phe Pro
 30                 55                   60                   65

        Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile
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        Ser Ile Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His
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    (2) INFORMATION FOR SEQ ID NO:3:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 1954 base pairs
 40   (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: cDNA to mRNA

      (iii) HYPOTHETICAL: NO

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        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
             (A) ORGANISM: Homo sapiens
             (G) CELL TYPE: Osteosarcoma Cell Line
             (H) CELL LINE: U-2OS

       (vii) IMMEDIATE SOURCE:
             (A) LIBRARY: U2OS cDNA in Lambda gt10
             (B) CLONE: Lambda U2OS-3

      (viii) POSITION IN GENOME:
             (C) UNITS: bp


        (ix) FEATURE:
             (A) NAME/KEY: CDS
             (B) LOCATION: 403..1629

        (ix) FEATURE:
             (A) NAME/KEY: mat_peptide
             (B) LOCATION: 1279..1626

        (ix) FEATURE:
             (A) NAME/KEY: mRNA
             (B) LOCATION: 9..1934


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC   180

 ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG   240

 CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC   300

 GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA   360

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                                               -292     -290

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         -270               -265               -260

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 Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu
     -255               -250               -245

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Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr
        -190                -185                -180

GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC      798
Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr
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AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT      846
Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro
-160                -155                -150                -145

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Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln
                -140                -135                -130

GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT      942
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TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC      990
Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile
        -110                -105                -100

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        -95                 -90                 -85

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-80                 -75                 -70                 -65

CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT     1134
Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr
                -60                 -55                 -50

CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA     1182
Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln
            -45                 -40                 -35

GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC ACC TTT GGC     1230
Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val Thr Phe Gly
        -30                 -25                 -20

CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG CGT     1278
His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys Arg
        -15                 -10                 -5

AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC     1326
Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys
    1               5                   10                  15

CGG CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC     1374
Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp
            20                  25                  30

TGG ATT GTG GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC     1422
Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp
        35                  40                  45

TGC CCC TTT CCA CTG GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT     1470
Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
    50                  55                  60
```

41

```
        GTG CAG ACC CTG GTC AAT TCT GTC AAT TCC AGT ATC CCC AAA GCC TGT    1518
        Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys
         65                  70                  75                  80

        TGT GTG CCC ACT GAA CTG AGT GCC ATC TCC ATG CTG TAC CTG GAT GAG    1566
 5      Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu
                         85                  90                  95

        TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG ATG GTA GTA GAG GGA    1614
        Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly
                    100                 105                 110

10      TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC       1666
        Cys Gly Cys Arg
                    115
```

CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC   1726

ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA   1786

ATCCCTAAAC ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT   1846

TGATCATATA TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG   1906

AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC   1954

        (2) INFORMATION FOR SEQ ID NO:4:

                (i) SEQUENCE CHARACTERISTICS:
20   (A) LENGTH: 408 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: protein

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25   Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val
        -292   -290   -285   -280

        Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys
            -275   -270   -265

        Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly
30   -260   -255   -250   -245

        Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met
                    -240   -235   -230

        Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro
                    -225   -220   -215

35   Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu
                -210   -205   -200

        Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser
            -195   -190   -185

        Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn
40   -180   -175   -170   -165

        Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu
                    -160   -155   -150

        Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu
                    -145   -140   -135
```

```
        Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His
            -130                -125                -120
        Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro
            -115                -110                -105
   5    Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn
        -100                -95                 -90                 -85
        Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp
                    -80                 -75                 -70
        Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His
   10           -65                 -60                 -55
        Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg
                -50                 -45                 -40
        Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu
            -35                 -30                 -25
   15   Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg
        -20                 -15                 -10                 -5
        Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys
                        1                   5                   10
        Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
   20           15                  20                  25
        Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
            30                  35                  40
        Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
        45                  50                  55                  60
   25   Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
                        65                  70                  75
        Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
                    80                  85                  90
        Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
   30           95                  100                 105
        Val Val Glu Gly Cys Gly Cys Arg
        110                 115
```

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
   35   (A) LENGTH: 15 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA to mRNA

   40   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        CATGGGCAGC TCGAG   15

        (2) INFORMATION FOR SEQ ID NO:6:

              (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 34 base pairs

```
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: double
                    (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: cDNA to mRNA

5        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGCAGGCGA GCCTGAATTC CTCGAGCCAT CATG                                  34

    (2) INFORMATION FOR SEQ ID NO:7:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 68 base pairs
10            (B) TYPE: nucleic acid
              (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: cDNA to mRNA

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

15  CGAGGTTAAA AAACGTCTAG GCCCCCCGAA CCACGGGGAC GTGGTTTTCC TTTGAAAAAC     60

ACGATTGC                                                              68

    (2) INFORMATION FOR SEQ ID NO:8:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 470 base pairs
20            (B) TYPE: nucleic acid
              (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: DNA (genomic)

        (iii) HYPOTHETICAL: NO

25        (v) FRAGMENT TYPE: C-terminal

         (vi) ORIGINAL SOURCE:
              (A) ORGANISM: Homo sapiens
              (H) CELL LINE: W138 (genomic DNA)

        (vii) IMMEDIATE SOURCE:
30            (A) LIBRARY: human genomic library
              (B) CLONE: lambda 111-1

       (viii) POSITION IN GENOME:
              (C) UNITS: bp

         (ix) FEATURE:
35            (A) NAME/KEY: exon
              (B) LOCATION: 1..470

         (ix) FEATURE:
              (A) NAME/KEY: CDS
              (B) LOCATION: 1..456

40       (ix) FEATURE:
              (A) NAME/KEY: mat_peptide
              (B) LOCATION: 124..453

         (ix) FEATURE:
              (A) NAME/KEY: mRNA
45            (B) LOCATION: 1..470
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
     TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG      48
         Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
     -41 -40             -35             -30

 5   CAG GTG AGA GCA GTC ACG AGG AGG ACA CGG ATG GCG CAC GTG GCT GCG      96
     Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
     -25             -20             -15                     -10

     GGG TCG ACT TTA GCC AGG CGG AAA AGG AGC GCC GGG GCT GGC AGC CAC     144
     Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
10                   -5               1               5

     TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC     192
     Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
               10              15              20

     AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC     240
15   Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
           25              30              35

     GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT     288
     Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
     40              45              50                      55

20   ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG     336
     Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                     60              65              70

     GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG     384
     Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
25               75              80              85

     GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC     432
     Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
                 90              95              100

     GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG              .        470
30   Val Ala Glu Cys Gly Cys Arg
         105                 110
```

(2) INFORMATION FOR SEQ ID NO:9:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 150 amino acids
35   (B) TYPE: amino acid
              (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: protein

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
     Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
40   -41 -40             -35             -30

     Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
     -25             -20             -15                     -10


     Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                     -5               1               5

45   Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
               10              15              20
```

45

```
     Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
         25                  30              35

     Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
     40                  45              50                  55

5    Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                 60              65                  70

     Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
                 75              80                  85

     Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
10           90              95                  100

     Val Ala Glu Cys Gly Cys Arg
         105                  110
```

What is claimed is:

1.   A BMP-9 polypeptide comprising the amino acid sequence from amino acid #8 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).

2.   A BMP-9 polypeptide comprising the amino acid sequence from
5   amino acid #1 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).

3.   A BMP-9 polypeptide of claim 1 wherein said polypeptide is a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #8 - 110 of FIG. 3 (SEQ ID NO: 9).

4.   A BMP-9 polypeptide of claim 2 wherein said polypeptide is
10   a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #1-110 of FIG. 3. (SEQ ID NO: 9).

5.   A purified BMP-9 protein produced by the steps of
      (a) culturing a cell transformed with a cDNA comprising the nucleotide sequence from nucleotide #124 to #453 as shown in FIG.
15   3 (SEQ ID NO: 8); and
      (b) recovering and purifying from said culture medium a protein comprising the amino acid sequence from amino acid #1 to amino acid #110 as shown in FIG. 3 (SEQ ID NO: 9).

6.   A purified BMP-9 protein produced by the steps of
20   (a) culturing a cell transformed with a cDNA comprising the nucleotide sequence from nucleotide #124 to #453 as shown in FIG. 3 (SEQ ID NO: 8); and
      (b) recovering form said culture medium a protein comprising an amino acid sequence from amino acid #8 to amino
25   acid #110 as shown in Figure 3 (SEQ ID NO: 9).

7.   A BMP-9 protein characterized by the ability to induce the formation of cartilage and/or bone.

8.   A DNA sequence encoding a BMP-9 protein.

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9.   The DNA sequence of claim 8 wherein said DNA comprises

     (a)   nucleotide 124 to 453 (SEQ ID NO: 8); and

     (b)   sequences which hybridize thereto under stringent
hybridization conditions and exhibit the ability to form
cartilage and/or bone.

10.   The DNA sequence of claim 8 wherein said DNA comprises

     (a)   nucleotide 145 to 453 (SEQ ID NO: 8); and

     (b)   sequences which hybridize thereto under stringent
hybridization conditions and exhibit the ability to form
cartilage and/or bone.

11.   A host cell transformed with a DNA sequence encoding BMP-8.

12.   A method for producing a purified BMP-9 protein said method
comprising the steps of

     (a) culturing a cell transformed with a cDNA comprising the
nucleotide sequence encoding a BMP-9 protein; and

     (b) recovering and purifying said BMP-9 protein from the
culture medium.

13.   A pharmaceutical composition comprising an effective amount
of a BMP-9 protein in admixture with a pharmaceutically
acceptable vehicle.

14.   A composition of claim 13 further comprising a matrix for
supporting said composition and providing a surface for bone
and/or cartilage growth.

15.   The composition of claim 14 wherein said matrix comprises
a material selected from the group consisting of hydroxyapatite,
collagen, polylactic acid and tricalcium phosphate.

16.   A method for inducing bone and/or cartilage formation in a
patient in need of same comprising administering to said patient
an effective amount of the composition of claim 13.

17. A pharmaceutical composition for wound healing and tissue repair said composition comprising an effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle.

18. A method for treating wounds and/or tissue repair in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 17.

19. A purified mammalian BMP-9 protein produced by the steps of

(a)   culturing a cell transformed with (i) a DNA comprising the nucleotide sequence from nucleotide #610 to #1893 of SEQ ID NO:1 and (ii) sequences which hybridize thereto under stringent hybridization conditions and induces the formation of cartilage or bone; and

(b)   recovering and purifying from said culture medium a protein comprising amino acid #1 to #110 of SEQ ID NO:9.

20. A pharmaceutical composition for hepatocyte growth said composition comprising an effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle.

21. A method for inducing hepatocyte growth in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 20.

22. A pharmaceutical composition for cartilage repair said composition comprising an effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle.

Figure 1/1

```
                10         20         30         40         50         60         70
        CATTAATAAA TATTAAGTAT TGGAATTAGT GAAATTGGAG TTCCTTGTGG AAGGAAGTGG GCAAGTGAGC
                80         90        100        110        120        130        140
        TTTTTAGTTT GTGTCGGAAG CCTGTAATTA CGGCTCCAGC TCATAGTGGA ATGGCTATAC TTAGATTTAT
               150        160        170        180        190        200        210
        GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG CCTAGGAGAT TTGTTGATCC AATAAAATATG
               220        230        240        250        260        270        280
        ATTAGGGAAA CAATTATTAG GGTTCATGTT CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTTTGATAA
               290        300        310        320        330        340        350
        TAAGTTTAAC TAGTCAGTGT TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGGAATAG
               360        370        380        390        400        410        420
        GATTGAAGGA AATATAAATGA TGGCTACAAC GATTGGGAAT CCTATTATTG TTGGGGTAAT GAATGAGGCA
               430        440        450        460        470        480        490
        AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA TTGGTGAGTA
               500        510        520        530        540        550        560
        GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA CCTGATGTTA GAAGGAGGGC
               570        580        590        600        609                  618
        TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG GCCACCCGG  ATG TCC CCT GGG
                                                              M   S   P   G
```

Figure 1/2

```
      627         636         645         654         663         672
    | GCC TTC CGG GTG GCC CTG CTC CCG CTG TTC CTG CTG GTC TGT GTC ACA CAG CAG
      A   F   R   V   A   L   L   P   L   F   L   L   V   C   V   T   Q   Q

      681         690         699         708         717         726
    | AAG CCG CTG CAG AAC TGG GAA CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC
      K   P   L   Q   N   W   E   Q   A   S   P   G   E   N   A   H   S   S

      735         744         753         762         771         780
    | CTG GGA TTG TCT GGA GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG
      L   G   L   S   G   A   G   E   E   G   V   F   D   L   Q   M   F   L

      789         798         807         816         825         834
    | GAG AAC ATG AAG GTG GAT TTC CTA CGC AGC AGC CTT AAC CTC AGC GGC ATT CCC TCC
      E   N   M   K   V   D   F   L   R   S   S   L   N   L   S   G   I   P   S
```

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Figure 1/3

```
       843       852       861       870       879       888
       CAG GAC AAA ACC AGA GCG GAG CCA CCC CAG TAC ATG ATC GAC TTG TAC AAC AGA
        Q   D   K   T   R   A   E   P   P   Q   Y   M   I   D   L   Y   N   R

       897       906       915       924       933       942
       TAC ACA ACG GAC AAA TCG TCT ACG CCT GCC TCC AAC ATC GTG CGG AGC TTC AGC
        Y   T   T   D   K   S   S   T   P   A   S   N   I   V   R   S   F   S

       951       960       969       978       987       996
       GTG GAA GAT GCT ATA TCG ACA GCT GCC ACG GAG GAC TTC CCC TTT CAG AAG CAC
        V   E   D   A   I   S   T   A   A   T   E   D   F   P   F   Q   K   H

       1005      1014      1023      1032      1041      1050
       ATC CTG ATC TTC AAC ATC TCC ATC CCG AGG CAC GAG CAG ATC ACC AGG GCT GAG
        I   L   I   F   N   I   S   I   P   R   H   E   Q   I   T   R   A   E
```

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Figure 1/4

```
1059      1068      1077      1086      1095      1104

CTC CGA CTC TAT GTC TCC TGC CAA AAT GAT GTG GAC TCC ACT CAT GGG CTG GAA
 L   R   L   Y   V   S   C   Q   N   D   V   D   S   T   H   G   L   E

1113      1122      1131      1140      1149      1158

GGA AGC ATG GTC GTT TAT GAT GTT CTG GAG GAC AGT GAG ACT TGG GAC CAG GCC
 G   S   M   V   V   Y   D   V   L   E   D   S   E   T   W   D   Q   A

1167      1176      1185      1194      1203      1212

ACG GGG AAG ACC TTC TTG GTA TCC CAG GAC ATT CGG GAC GAA GGA TGG GAG
 T   G   K   T   F   L   V   S   Q   D   I   R   D   E   G   W   E

1221      1230      1239      1248      1257      1266

ACT TTA GAA GTA TCG AGT GCC GTG AAG CGG TGG GTC AGG GCA GAC TCC ACA ACA
 T   L   E   V   S   S   A   V   K   R   W   V   R   A   D   S   T   T
```

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Figure 1/5

```
      1275        1284        1293        1302        1311        1320
AAC | AAA AAT AAG | CTC GAG GTG | ACA CAG AGC | CAC AGG GAG | AGC TGT GAC | ACA
 N  |  K   N   K  |  L   E   V  |  T   Q   S  |  H   R   E  |  S   C   D  |  T

      1329        1338        1347        1356        1365        1374
CTG | GAC ATC AGT | GTC CCT CCA | GGT TCC AAA | AAC CTG CCC | TTC TTT GTT | GTC TTC
 L  |  D   I   S  |  V   P   P  |  G   S   K  |  N   L   P  |  F   F   V  |  V   F

      1383        1392        1401        1410        1419        1428
TCC | AAT GAC CGC | AGC AAT GGG | ACC AAG GAG | ACC AGA CTG | GAG CTG AAG | GAG ATG
 S  |  N   D   R  |  S   N   G  |  T   K   E  |  T   R   L  |  E   L   K  |  E   M

      1437        1446        1455        1464        1473        1482
ATC | GGC CAT GAG | CAG GAG ACC | ATG CTT GTG | AAG ACA GCC | AAA AAT GCT | TAC CAG
 I  |  G   H   E  |  Q   E   T  |  M   L   V  |  K   T   A  |  K   N   A  |  Y   Q
```

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Figure 1/6

```
       1491      1500      1509      1518      1527      1536
       GTG GCA | GGT GAG AGC | CAA GAG GAG GAG | GGT CTA GAT GGA | TAC ACA GCT GTG | GGA
        V   A     G   E   S     Q   E   E   E     G   L   D   G     Y   T   A   V     G

       1545      1554      1563      1572      1581      1590
       CCA CTT | TTA GCT AGA AGG | AAG AGC ACC GGA | GCC AGC CAC TGC | CAG AAG
        P   L     L   A   R   R     K   S   T   G     A   S   H   C     Q   K
                               (319)                                (326)

       1599      1608      1617      1626      1635      1644
       ACT TCT | CTC AGG GTG AAC | TTT GAG GAC ATC | GGC TGG GAC AGC | TGG ATC ATT GCA
        T   S     L   R   V   N     F   E   D   I     G   W   D   S     W   I   I   A

       1653      1662      1671      1680      1689      1698
       CCC AAG | GAA TAT GAC GCC | TAT GAG TGT AAA | GGG GGT TGC TTC | TTC CCA TTG GCT
        P   K     E   Y   D   A     Y   E   C   K     G   G   C   F     F   P   L   A
```

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Figure 1/7

```
     1707      1716      1725      1734      1743      1752
     |         |         |         |         |         |
GAT GAC  GTG ACA CCC  AAA CAT GCC  ATC GTG CAG  ACC CTG GTG  CAT CTC GAG
 D   D    V   T   P    K   H   A    I   V   Q    T   L   V    H   L   E

     1761      1770      1779      1788      1797      1806
     |         |         |         |         |         |
TTC CCC  ACA AAG GTG  GGC AAA GCC  TGC TGC GTT  CCC ACC AAA  CTG AGT CCC ATC
 F   P    T   K   V    G   K   A    C   C   V    P   T   K    L   S   P   I

     1815      1824      1833      1842      1851      1860
     |         |         |         |         |         |
TCC ATC  CTC TAC AAG  GAT GAC ATG  GGG GTG CCA  ACC CTC AAG  TAC CAC TAT GAG
 S   I    L   Y   K    D   D   M    G   V   P    T   L   K    Y   H   Y   E

     1869      1878      1887      1903      1913      1923
     |         |         |              ^
GGG ATG AGT  GTG GCT GAG  TGT GGG  TGT AGG TAGTCCCTGC AGCCACCCAG GGTGGGGATA
 G   M   S    V   A   E    C   G    C   R
                                      (428)
```

Figure 1/8

```
       1933       1943       1953       1963       1973       1983       1993
CAGGACATGG AAGAGGTTCT GGTACGGTCC TGCATCCTCC TGCGCATGGT ATGCCTAAGT TGATCAGAAA
       2003       2013       2023       2033       2043       2053       2063
CCATCCTTGA GAAGAAAAGG AGTTAGTTGC CCTTCTTGTG TCTGGTGGGT CCCTCTGCTG AAGTGACAAT
       2073       2083       2093       2103       2113       2123       2133
GACTGGGGTA TGCGGGCCTG TGGGCAGAGC AGGAGACCCT GGAAGGGTTA GTGGGTAGAA AGATGTCAAA
       2143       2153       2163       2173       2183       2193       2203
AAGGAAGCTG TGGGTAGATG ACCTGCACTC CAGTGATTAG AAGTCCAGCC TTACCTGTGA GAGAGCTCCT
       2213       2223       2233       2243       2253       2263       2273
GGCATCTAAG AGAACTCTGC TTCCTCATCA TCCCCACCGA CTTGTTCTTC CTTGGGGAGTG TGTCCTCAGG
       2283       2293       2303       2313       2323       2333       2343
GAGAACAGCA TTGCTGTTCC TGTGCCTCAA GCTCCCAGCT GACTCTCCTG TGGCTCATAG GACTGAATGG
       2353       2363       2373       2383       2393       2403       2413
GGTGAGGAAG AGCCTGATGC CCTCTGGCAA TCAGAGCCCG AAGGACTTCA AAACATCTGG ACAACTCTCA
       2423       2433       2443
TTGACTGATG CTCCAACATA ATTTTTAAAA AGAG
```

Figure 2/1

```
        10          20          30          40          50          60          70
CTCTAGAGGG  CAGAGGAGGA  GGGAGGGAGG  GAAGGGAGCGC  GGAGCCCGGC  CCGGAAGCTA  GGTGAGTGTG

        80          90         100         110         120         130         140
GCATCCGAGC  TGAGGGACGC  GAGCCTGAGA  CGCCGCTGCT  GCTCCGGCTG  AGTATCTAGC  TTGTCTCCCC

       150         160         170         180         190         200         210
GATGGGATTC  CCGTTCCAAGC  TATCTCGAGC  CTGCAGCGCC  ACAGTCCCCG  GCCCTCGCCC  AGGTTCACTG

       220         230         240         250         260         270         280
CAACCGTTCA  GAGGTCCCCA  GGAGCTGCTG  CTGGCCGAGCC  CGCTACTGCA  GGGACCTATG  GAGCCATTCC

       290         300         310         320         330         340         350
GTAGTGCCAT  CCCGAGCAAC  GCACTGCTGC  AGCCTTCCCTG  AGCCTTTCCA  GCAAGTTTGT  TCAAGATTGG

       360         370         380         390         400
CTGTCAAGAA  TCATGGACTG  TTATTATATG  CCTTGTTTTC  TGTCAAGACA  CC ATG ATT CCT
                                                   (1)
                                                        MET Ile Pro

  417                   432                        447                462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala
```

Figure 2/2

```
     477              492              507
AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

     522              537              552              567
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

     582              597              612              627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CCG CAG CCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Pro Gln Pro Ser Lys

     642              657              672
AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

     687              702              717              732
GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC
Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala
```

Figure 2/3

```
                747              762              777
AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792              807              822              837
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

852              867              882              897
CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG GAG CTC TTC CGG GAG CAG GTG
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Glu Leu Phe Arg Glu Gln Val

912              927              942
GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957              972              987              1002
ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
```

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Figure 2/4

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         1017              1032              1047
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

  1062              1077              1092              1107
GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

         1122              1137              1152              1167
GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

                 1182              1197              1212
CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

  1227              1242              1257              1272
ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG
Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys

         1287              1302              1317
CGT AGC CCT AAG CAT CAC TCA CAG GCC CGG AAG AAG AAT AAG AAC TGC CGG
Arg Ser Pro Lys His His Ser Gln Ala Arg Lys Lys Asn Lys Asn Cys Arg
```

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1332(311)   1347   1362   1377
CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

          1392   1407   1422   1437
GCC CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

          1452   1467   1482
GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

          1497   1512   1527   1542
GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

          1557   1572   1587
TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

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1602                1617          (408)     1636      1646      1656
ATG GTA GTA GAG GGA GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
MET Val Val Glu Gly Gly Cys Gly Cys Arg

       1666      1676      1686      1696      1706      1716      1726
ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC

       1736      1746      1756      1766      1776      1786      1796
ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC

       1806      1816      1826      1836      1846      1856      1866
ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA

       1876      1886      1896      1906      1916      1926      1936
ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT

       1946
CTAGAGTCGA CGGAATTC
```

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Figure 3

```
TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG    48
 *  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41     -40              -35              -30

CAG GTG AGA GCA GTC ACG AGG ACA CGG ATG GCG CAC GTG GCT GCG        96
Gln Val Arg Ala Val Arg Thr Arg Arg Met Ala His Val Ala Ala
-25         -20              -15              -10

GGG TCG ACT TTA GCC AAA AGG AGC GCC GGG GCT GGC AGC CAC           144
Gly Ser Thr Leu Ala Lys Arg Ser Ala Gly Ala Gly Ser His
         -5               1               5

TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC   192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
         10              15              20

AGC TGG ATC ATT GCA CCC TAT GAA GCC TAC TGT GAG AAG GGC           240
Ser Trp Ile Ile Ala Pro Tyr Glu Ala Tyr Cys Glu Lys Gly
         25              30              35

GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG AAA CAC GCT       286
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Lys His Ala
         40              45              50              55

ATC GTG CAG ACC CTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG       336
Ile Val Gln Thr Leu His Leu Lys Phe Pro Thr Lys Val Gly Lys
         60              65              70

GCC TGC ACC AAA CTG AGC CCC ACC AAA ATC TCC GTC CTC TAC AAG       384
Ala Cys Thr Lys Leu Ser Pro Thr Lys Ile Ser Val Leu Tyr Lys
         75              80              85

GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC   432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
         90              95              100
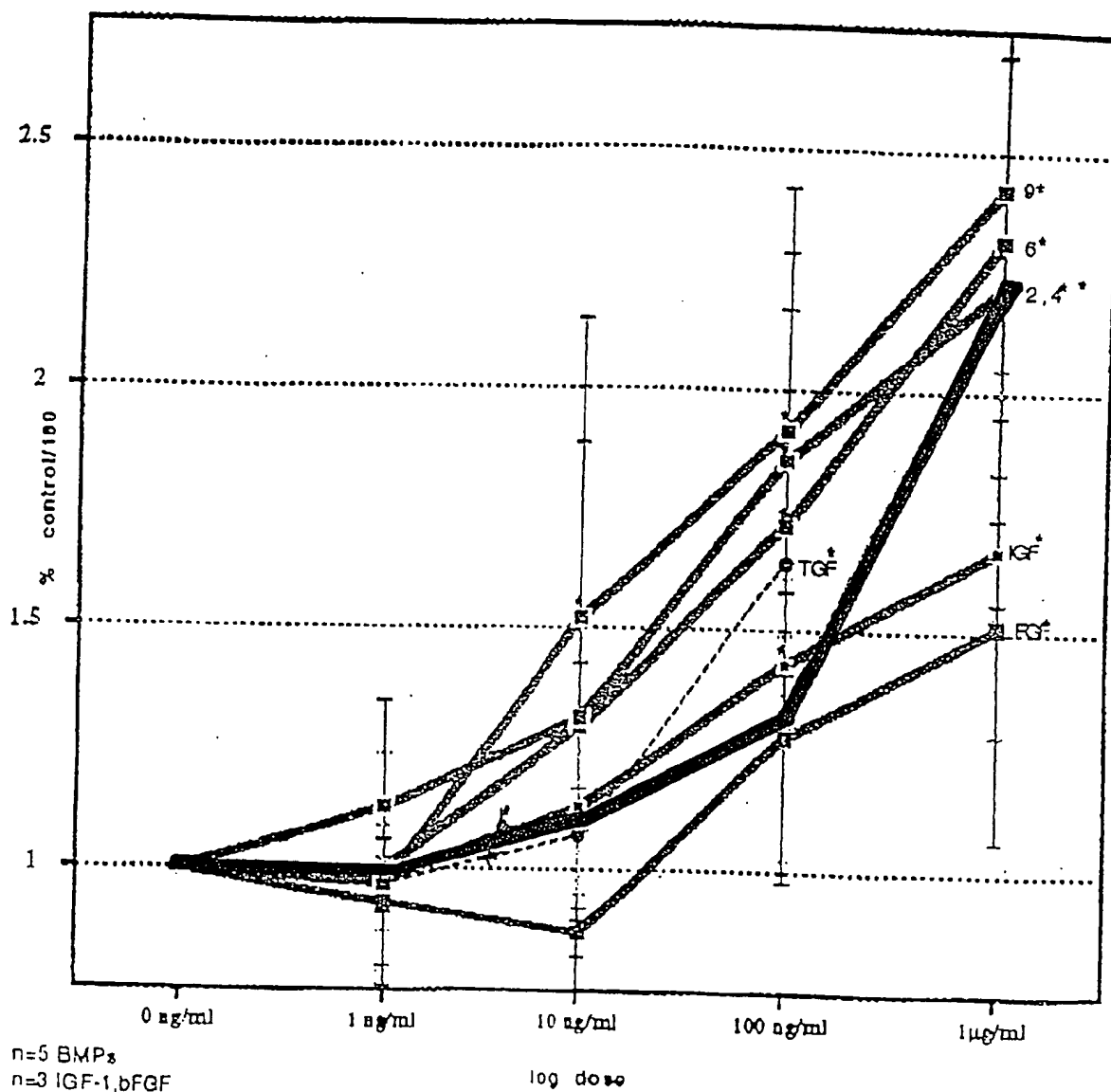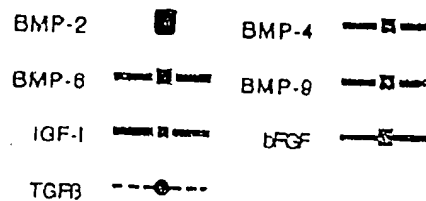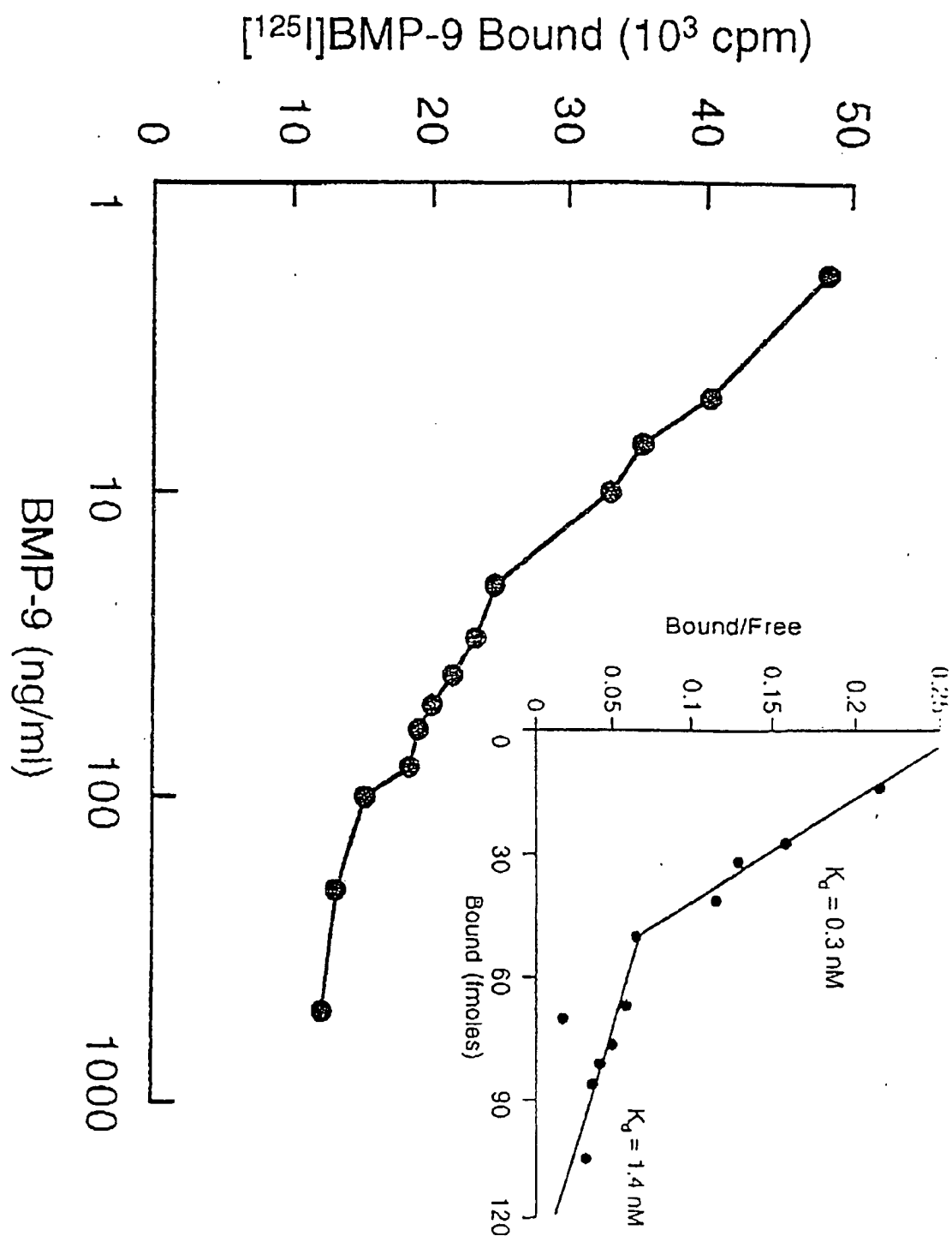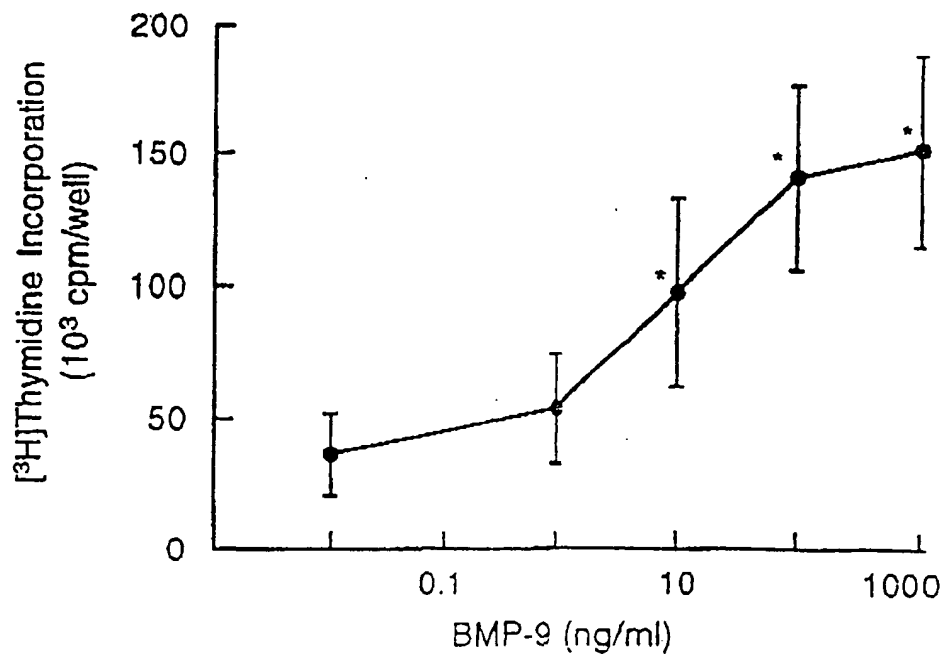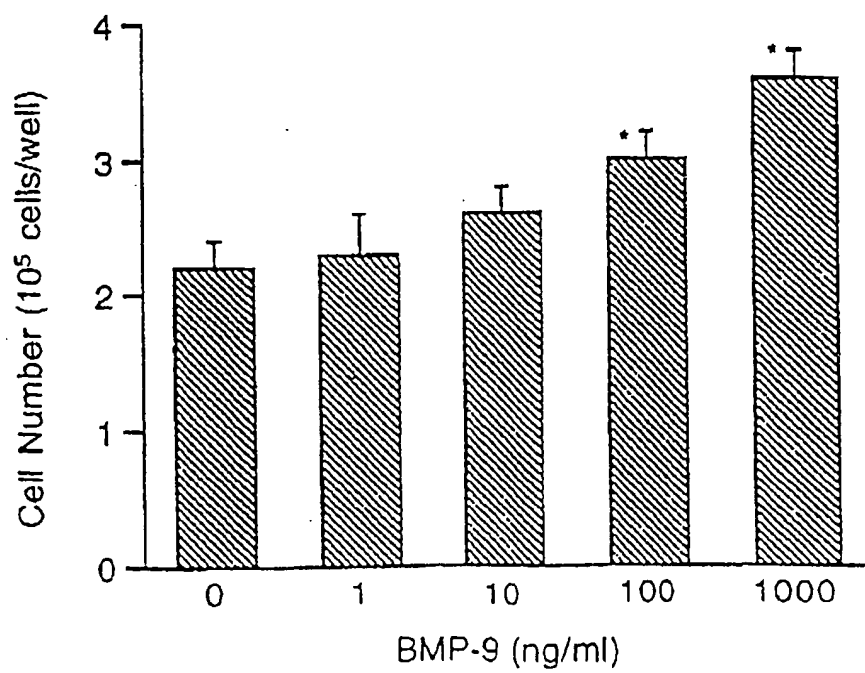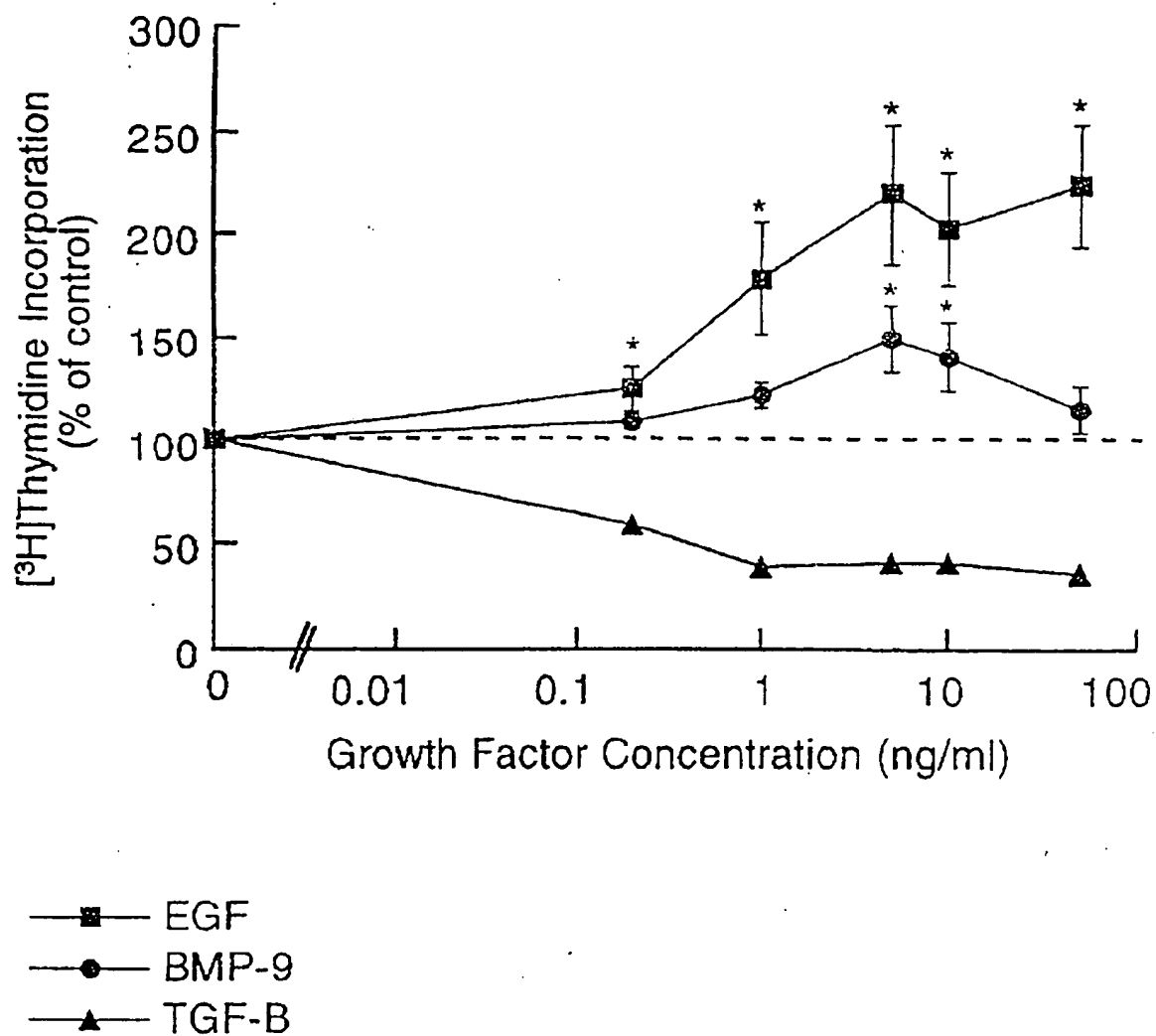
GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG                    470
Val Ala Glu Cys Gly Cys Arg
         105             110
```

Figure 4

SULFATE INCORPORATION

Bovine Explants

n=5 BMPs
n=3 IGF-1,bFGF
n=1 TGFB
*=different than control (p<0.05)

BMP-2   BMP-4
BMP-6   BMP-9
IGF-1   bFGF
TGFB

Figure 5

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Figure 6/2

SUBSTITUTE SHEET (RULE 26)

Figure 7

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/12   C07K14/51   A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07K   C12N   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 00432 (GENETICS INST) 7 January 1993	7,8, 11-18,22
Y	see the whole document ---	20,21
Y	WO,A,94 06449 (CREATIVE BIOMOLECULES INC) 31 March 1994 see the whole document --- -/--	20,21

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 1995	16.11.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Andres, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,P, X	MOLECULAR BIOLOGY OF THE CELL, vol. 5, October 1994 page 384a SONG, J. ET AL. 'Bone morphogenetic protein-9 (BMP-9) binds to HEPG2 cells and stimulates proliferation' see abstract & 34th Ann.Meet. of the American Soc. for Cell Biol.; december 10-14, 1994; San Francisco, California	20,21

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 95/ 07084

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 16,18,21 are directed to a method of treatment of

   the human/animal body, the search has been carried out and based on the
   aaleged effects of the compound/composition.

2. [X] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:
   Claim 11 refering to a DNA sequence encoding BMP-8, has been interpreted
   as being meant to refer to BMP-9!

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all
   searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9300432	07-01-93	AU-B-	652472	25-08-94
		AU-A-	2269992	25-01-93
		EP-A-	0592562	20-04-94
		JP-T-	6508990	13-10-94
WO-A-9406449	31-03-94	AU-B-	4795193	03-03-94
		AU-B-	4797193	03-03-94
		AU-B-	4995593	03-03-94
		AU-B-	5129293	12-04-94
		AU-B-	5129393	12-04-94
		AU-B-	5162393	12-04-94
		AU-B-	5290893	12-04-94
		AU-B-	5590094	24-05-94
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		CA-A-	2141555	17-02-94
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		EP-A-	0653942	24-05-95
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		EP-A-	0661987	12-07-95
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		WO-A-	9406447	31-03-94
		WO-A-	9406399	31-03-94
		WO-A-	9406420	31-03-94
		WO-A-	9410203	11-05-94 |